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# **TRANSFUSION IN CRITICALLY ILL PATIENTS: SHORT- AND LONG-TERM OUTCOMES**

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# Transfusion in critically ill patients: short- and long-term outcomes

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

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## 1. Abstract

A blood transfusion is a common treatment for a range of conditions. Over 112 million blood transfusions are administered each year worldwide and many patients are thereby exposed to the possible risks associated with the procedure. In this thesis we focus on the critically ill patient, who is both to a great extent exposed to blood transfusion, and by his/her underlying illness, also susceptible to its adverse effects. We aimed to describe the population of the massively transfused patients and study possible negative effects associated with certain parts of the transfusion therapies. Additionally, we investigated the possibility to identify, through national health registers, the rare, but serious condition transfusion related acute lung injury (TRALI) which today is considered the leading cause of transfusion-related mortality. In the first study we characterized the population of massively transfused patients in Sweden and Denmark during the last decades. We found a non-negligible incidence of massive transfusion with the dominating indication being major surgery. The overall mortality among massively transfused patients was high, both expressed as 30-day- and 5-year-mortality. The standardized mortality ratio (SMR) was 26.2 during the first 6 months after transfusion and decreased gradually with time but was still elevated as long as 10 years after the transfusion event. In the second study, we studied the effect of plasma to red blood cell ratio among bleeding trauma patients. With a time-dependent model, and in contrast to previous observational data, we found no difference in outcome between high and low plasma ratio. We suggest that previous research suffered from severe bias and conclude that no strong evidence for using high plasma ratio in trauma patients exists today. Our third study investigated a possible detrimental effect of the storage time of red blood cells. We used three different analytical approaches to assess the association between storage time of red blood cells and mortality in transfused patients. Consistently, throughout all analyses, we found no such association. Our results, which are concordant with recently published randomized controlled trials, indicate the safety of today's practice to store red blood cells for up to 42 days. The fourth study was performed with the aim to develop and test a statistical method for identifying donors with high risk of causing TRALI in the recipient. The statistical method was based on the diagnosis of acute respiratory distress syndrome (ARDS) among transfused patients. We constructed a risk score for each donor based on the difference between observed and expected ARDS cases among that donor's recipients. Through this risk score we selected patients for manual review of medical records. The review resulted in identification of only one definitive TRALI case and we conclude that our statistical method, for the moment, fails to be a way of identifying and further study the condition.

## 2. List of publication

This thesis includes the following four publications. The publications are here reproduced with permission of the publishers.

1. Märit Halmin; Flaminia Chiesa; Senthil K. Vasan; Agneta Wikman; Rut Norda; Klaus Rostgaard; Ole Birger Vesterager Pedersen; Christian Erikstrup; Kaspar René Nielsen; Kjell Titlestad; Henrik Ullum; Henrik Hjalgrim; Gustaf Edgren. *Epidemiology of Massive Transfusion: a Binational Study from Sweden and Denmark*. Critical Care Medicine 2016; 44:468-77.
2. Märit Halmin; Fredrik Boström; Olof Brattström; Joachim Lundahl; Agneta Wikman, MD; Anders Östlund; Gustaf Edgren. *Effect of Plasma-to-RBC Ratios in Trauma Patients: a Cohort Study with Time-Dependent Data*. Critical Care Medicine 2013; 41:1905-14.
3. Märit Halmin; Klaus Rostgaard; Brian K. Lee; Agneta Wikman; Rut Norda; Kaspar Rene Nielsen; Ole B. Pedersen; Jacob Holmqvist; Henrik Hjalgrim; Gustaf Edgren. *Length of storage of red blood cells and patient survival after blood transfusion: a bi-national cohort study*. Ann Int Med 2016, 20 of December. Epub ahead of print.
4. Märit Halmin; Agneta Wikman; Rut Norda; Henrik Ullum; Kjell Titlestad; Ole Pedersen; Klaus Rostgaard; Henrik Hjalgrim; Gustaf Edgren. *Clustering of acute respiratory distress syndrome: a statistical approach for studying transfusion-related acute lung injury*. Submitted Manuscript.

### 3. Table of contents

1. Abstract .....	2
2. List of publication .....	3
3. Table of contents .....	4
4. Abbreviations .....	7
5. Introduction.....	9
6. Background.....	10
6.1 History .....	10
6.2 Importance for society .....	11
6.3 Bleeding/massive bleeding.....	11
6.4 Epidemiology .....	12
6.5 Physiology.....	13
6.6 Early Trauma Induced Coagulopathy .....	13
6.7 Treatment.....	14
6.8 The proportion of blood components in transfusion.....	15
6.9 Adverse reactions.....	16
6.10 Transfusion Related Acute Lung Injury.....	18
6.11 Storage time .....	20
6.12 Methodological problems .....	21
7. Specific aims .....	23
8. Methods and materials .....	24
8.1 Data sources .....	24
8.1.1 SCANDAT database.....	24
8.1.2 Patient register .....	25
8.1.3 Regional trauma register .....	26
8.1.4 Medical records .....	26
8.2 Study Designs .....	26
8.2.1 Study 1.....	26
8.2.2 Study 2.....	27
8.2.3 Study 3.....	28
8.2.4 Study 4.....	29
8.3 Statistical analyses.....	30
8.3.1 Study 1.....	30
8.3.2 Study 2.....	30

8.3.3 Study 3 .....	31
8.3.4 Study 4 .....	32
8.4 Ethical considerations .....	33
9. Results .....	35
9.1 Study 1 .....	35
9.1.1 Study population .....	35
9.1.2 Incidence .....	36
9.1.3 Mortality analyses .....	36
9.1.4 Other results.....	37
9.2 Study 2 .....	37
9.2.1 Study population .....	37
9.2.2 Mortality analyses .....	38
9.2.3 Other results.....	39
9.3 Study 3 .....	39
9.3.1 Study population .....	39
9.3.2 Mortality analyses .....	40
9.3.3 Other results.....	41
9.4 Study 4 .....	41
9.4.1 Study population .....	41
9.4.2 Association of risk score and the risk of ARDS .....	41
9.4.3 Medical review of cases and controls .....	42
9.4.4 Other analyses .....	42
10. Methodological considerations .....	44
10.1 Observational vs randomized studies .....	44
10.2 Confounding by indication .....	45
10.3 Survival bias .....	46
10.4 Number of transfusions.....	46
10.5 Blood allocation.....	47
10.6 Misclassification .....	48
10.7 Classification of indications .....	49
10.8 Observer bias.....	50
10.9 Defining cohorts .....	50
10.10 Missing data .....	51
10.11 Residual confounding .....	51

11. Main findings and implications .....	54
11.1 Study 1.....	54
11.2 Study 2.....	54
11.3 Study 3.....	55
11.4 Study 4.....	55
12. Conclusions.....	56
13. Future perspectives.....	57
13.1 Blood replacement .....	57
13.2 Prediction of massive transfusion .....	57
13.3 Extending cohorts.....	57
13.4 Storage time, morbidity and efficacy .....	58
13.5 Whole blood transfusions .....	58
13.6 Long-term outcomes .....	58
13.7 Expanding SCANDAT2.....	59
13.8 Ratio vs goal-directed therapy .....	59
13.9 TRALI and TACO.....	60
14. Svensk sammanfattning .....	61
15. Acknowledgements .....	62
16. References.....	64



## 4. Abbreviations

ARDS	Acute Respiratory Distress Syndrome
BNP	Brain Natriuretic Peptide
CAT	Critical Administration Threshold
CRP	C-Reactive Protein
CVP	Central Venous Pressure
ER	Emergency Room
ETIC	Early Traumatic Induced Coagulopathy
GCS	Glasgow Coma Scale
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigens
HR	Hazard Ratio
ICD	International Classification of Disease
ICU	Intensive Care Unit
INR	International Normalized Ratio
IQR	Inter Quartile Range
ISS	Injury Severity Score
IV	Instrumental Variable
MOF	Multi Organ Failure
NEC	Necrotizing Enterocolitis
NRN	National Registration Number
OR	Odds Ratio
PAI-1	Plasminogen Activator Inhibitor -1
paO <sub>2</sub> /FiO <sub>2</sub>	Ratio of arterial oxygen partial pressure to fractional inspired oxygen
RBC	Red Blood Cell Concentrate
RCT	Randomized Controlled Trial
RhD	Rhesus D
SCANDAT	Scandinavian Donations and Transfusions Database
SIR	Swedish Intensive Care Register

SMR	Standardized Mortality Ratio
TACO	Transfusion Associated Circulatory Overload
TAGvH	Transfusion Associated Graft versus Host reaction
TRALI	Transfusion Related Acute Lung Injury
TRIM	Transfusion Related ImmunoModulation
US	United States
WB	Whole Blood
2,3 DPG	2,3 Diphosphoglycerate

## 5. Introduction

For clinicians managing critically ill patients, blood transfusions constitute an indisputable part of the treatment arsenal. However, to whom, when and how to transfuse is surrounded with many uncertainties. Correspondingly, even though we know about some adverse effects of blood transfusion, recognizing them and taking measures to prevent them is not always obvious. This is partly due to the complexity in treating critically ill patients, but also due to lack of knowledge among clinicians as well as paucity of scientific data. This thesis is firstly an effort to better describe and thereby understand the patients who are extremely exposed to blood transfusions, namely the massively transfused population. Secondly, the thesis focuses on two key questions in the transfusion treatment, the amount of plasma and the length of storage of red blood cells, and their possible influence on the patients' chance to survive. Finally, since the leading cause of transfusion-related death, TRALI, is distinctly hard to study and includes many doubts, this thesis explores a novel way of identifying the condition and studying risk factors associated primarily with the blood donors. Throughout history, there have always been efforts to discover replacements for blood transfusion, ranging from goat milk in the 19<sup>th</sup> century to the current development of synthetically produced hemoglobin derivatives, unfortunately a reasonable substitute still lies in the future. Therefore, the current approach must be focused on improvements of blood transfusion therapies in order to provide this immunological highly active product in the safest way possible. This thesis has the ambition to take one small step further in making blood transfusion as safe as possible.

## 6. Background

### 6.1 History

Everyone knows that blood is vital for human life. This knowledge goes far back in time recognizing the association between blood loss and severe illness or even death. Blood has also been surrounded by ideas of having magical power, carrying personality traits or being subject for contamination with demons and evil forces. Already during the Roman era blood loss, by opening veins, was used as a way to commit suicide and during the same era people drank blood from dead gladiators with the aim to gain their strength and courage. In the literature, vampires symbolize the importance of blood trying to achieve eternal life by drinking blood from a human being.

The circulation system was described in the 17<sup>th</sup> century and understanding that the heart was pumping out volumes of blood into the vessels changed the idea of replacing blood by drinking to instead infuse blood directly in the vessels. Initially transfusion was used to treat different states of madness or in order to gain positive properties from the person donating the blood. Not until 1749 was transfusion proposed as a specific treatment for bleeding conditions. Several scientists experimented with transfusions between animals and between animals and humans. Although some of these experiments succeeded, many animals and persons died and adverse reactions like fever, gastrointestinal symptoms and dark urine were described. Blundell, an English obstetrician, claimed that transfusions should take place within species and in 1829 he performed the first successful transfusion from human to human in a woman with severe post-partum hemorrhage. From then on practical obstacles surrounding transfusion was the major concern.

The first transfusions were made in a direct way by connection of the donor's artery to the vein of the recipient and thus letting the hydrostatic pressure drive the transfusion. A range of devices to facilitate this process was invented. Another barrier was the rapid coagulation of blood outside the body which complicated the procedure and various attempts to prevent this process was undertaken with varying results. Due to the obstacles surrounding blood transfusions, scientists focused on discovering alternative liquids that could replace blood. In the late 19<sup>th</sup> century, goat milk was the fluid of choice but the treatment was stopped quickly and unsurprisingly, due to severe adverse reactions (1, 2).

From the beginning of and throughout the 20<sup>th</sup> century, transfusion strategies have made huge advances. Karl Landsteiner discovered the ABO-system in 1901 and transfusion of compatible blood was introduced. This discovery was rewarded with the Nobel Prize in 1930 (3). In 1939 the same man, Landsteiner, also discovered the Rhesus system which further reduced previous transfusion reactions. Apart from testing for incompatibility, testing for possible transmittable disease has

evolved over the years and the introduction of tests for Hepatitis and HIV and advances in testing strategies have improved transfusion safety in a remarkable way. Today the risk of transmission of hepatitis- or HIV-virus by transfusion is less than 1 in 1 million units transfused (4). During the first decades of the 20<sup>th</sup> century refrigeration of blood as well as the discovery of different additives to prevent coagulation enabled storage of blood products. After the First World War, this became common practice. Indirect blood transfusion was implemented and used to a great extent during the Second World War (5, 6). Blood banks developed throughout the western world and since then development of additives and processing of whole blood into blood components, have advanced further up until today's praxis to store red blood cells for as long as 42 days, plasma as frozen units up to 3 years and platelets for seven days (7). In 1940, the ethanol fractionation was developed which enabled plasma to be broken down in different products such as albumin, immunoglobulins and fibrinogen which became available for clinical use. In 1970 apheresis, the method for extracting one cellular component of blood, or plasma, and returning the rest to the donor, was introduced (8). Nowadays the focus is on development projects regarding the indications and use of blood components, improvement of additive solutions for storage and development of protocols for pathogen reduction in order to improve safety.

## **6.2 Importance for society**

Today blood transfusion includes a range of different blood products and is used for a large variety of indications. More than 112 million blood transfusions are performed yearly over the world (9). Blood transfusion and its safety is of great concern for society since it involves a large part of its population both on the side of donors and recipients. At the age of 80 years as many as 20% of the general population have received at least one blood transfusion (10), and in any given year 3% of the Swedish adult population are active blood donors (11).

## **6.3 Bleeding/massive bleeding**

Although the majority of the blood transfusions performed today are limited to one or two red blood cell concentrates (RBC) (10), a considerable proportion of blood transfusions are administered to massively bleeding patients. This patient group is extremely exposed to potential risks associated with blood transfusion and are therefore of great interest when studying blood transfusion and its effects.

There is no universal definition of massive bleeding or massive transfusion, but the most commonly used definition is the administration of 10 RBC or more within 24 hours. This cut-off is highly arbitrary and the definition is surrounded with various problems. Firstly, the definition of massive transfusion is retrospective in its nature which makes inclusion of a study population hard to

perform. The majority of those who die due to bleeding die within 3 hours (12), making it hard for them to have time to receive 10 units of RBC and are therefore rarely included in research about massive transfusion.

Secondly, the possibility among clinicians to predict at an early stage who is going to be massively transfused is notably low, with a positive predictive value of 35% (13). Even when the blood loss is external, the visual assessment is not precise; small blood volumes tend to be overestimated while large blood volumes tend to be underestimated (14).

Thirdly, there is a lack of easily adoptable prediction tools. Various attempts have been made to predict who is going to get massively transfused, but until now no good prediction model exists and all proposed scoring systems have either been practically hard to use or suffered from low sensitivity and/or specificity (15). A consequence of the difficulty to predict massive transfusion is that patients, who are transfused but not enough to fulfill the criteria, will be excluded from the majority of studies. This might dilute certain effects and complicate the detection of clinically important results. Also, those who die from exsanguination before reaching the cut-off of 10 transfusions will not be included in retrospective studies and a potential beneficial effect on this group will be missed (15).

Finally, an intervention that possibly would result in reduced transfusion needs risks going undetected with the current definition.

To overcome the problems associated with the definition, Savage et.al (16) propose a modified definition, named Critical administration threshold (CAT). CAT is when a patient receives 3 units of RBC or more within one hour. Reaching this threshold defines the patient as CAT positive (CAT+). Every patient can be CAT+ up to 4 times within the study period. They claim that CAT+ better predicts mortality than the traditional definition of massive transfusion. Another study also showed that transfusion rates rather than a fixed number of transfusions should be used to predict mortality and reported a notable increase in mortality after administration of 4 units of RBC in one hour (17).

## **6.4 Epidemiology**

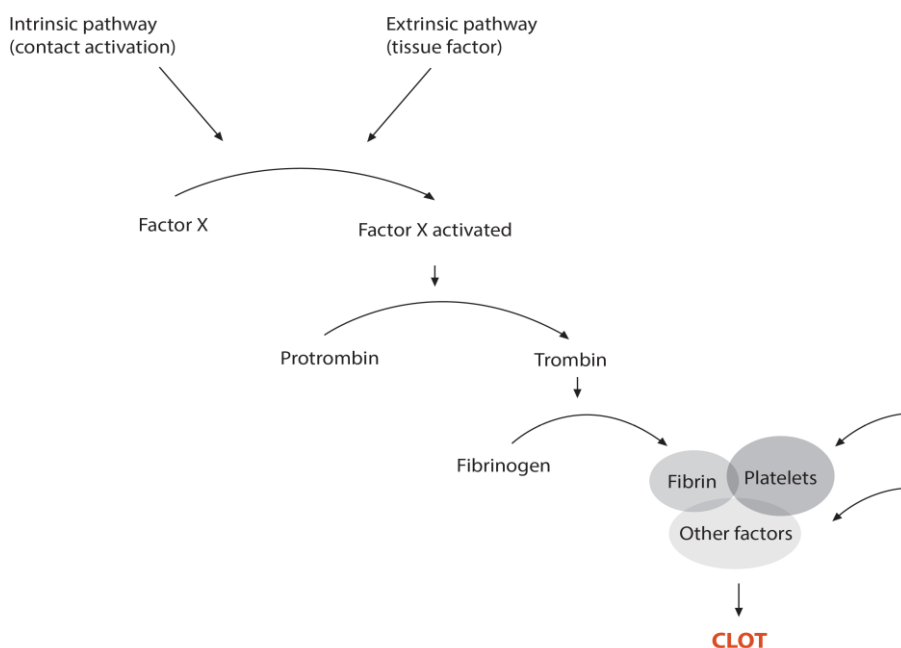
Even though there is some epidemiological research describing the general incidence of blood transfusion, the incidence of massive transfusion is largely unknown (18). Similarly, the indications and the mortality are unexplored. The overall perception is that it is mostly trauma and obstetric patients who are exposed to massive transfusion and research has therefore mainly focused on these specific patient groups.

## 6.5 Physiology

Bleeding is the loss of blood volume from the circulation and has large consequences for the human organism and its functions. First, the loss of red blood cells reduces the ability to transport oxygen and thereby decreasing the mandatory fuel for vital cellular processes. Secondly the loss of circulatory volume reduces tissue perfusion which further diminishes tissue oxygenation, and thirdly the loss of coagulation factors and platelets impairs the body's ability to stop the bleeding (19).

The physiological responses to injury and bleeding are many, one being the activation of the coagulation cascade (see Figure 1). Briefly this is mediated by an intrinsic and an extrinsic pathway. Both those pathways converge at the activation of clotting factor X which activates thrombin which in turn converts fibrinogen to fibrin. Fibrin then, in conjunction with platelets and other factors, produces a clot that prevents damaged tissue from continuing to bleed. After some time the clot is broken down by a process referred to as fibrinolysis, triggered by plasmin. The coagulation system is very complex, containing amplifying steps and a rigorous maintenance of balance between pro-coagulators and anti-coagulators is mandatory (20).

Figure 1. Coagulation cascade



## 6.6 Early Trauma Induced Coagulopathy

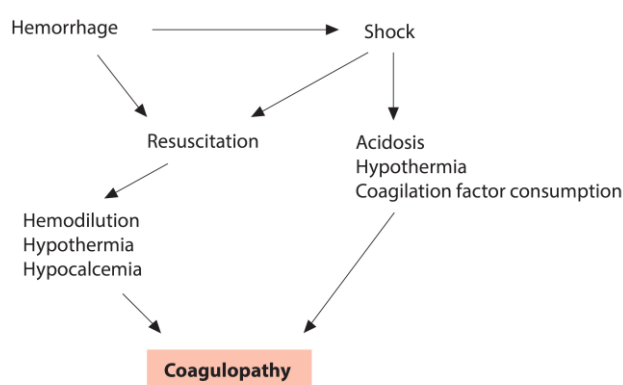
The leading cause of death among young persons is trauma (21). A large proportion of trauma related deaths are due to bleeding (22) and these deaths are in many cases considered to be preventable (21). Blood products play a main role in the treatment and account for approximately 1/3 of all costs associated with trauma care (23). In trauma patients an acquired coagulopathic

disorder, Early Traumatic Induced Coagulopathy (ETIC), has gained a lot of interest recently. ETIC is showed to develop within one hour from the injury and produces changes in coagulation parameters before any treatment has started. Among the most severely injured patients (with Injury Severity Score [ISS] > 25) the prevalence of ETIC is estimated to be 30% and the presence of ETIC is associated with increased mortality(24).The injury in itself is believed to trigger a range of alterations in the coagulation system that results in the coagulopathy characteristic for ETIC. Damaged endothelium expresses thrombomodulin that activates Protein C, which in turn stimulates plasmin formation and inhibits the action of Plasminogen Activator Inhibitory Factor-1 (PAI-1), both resulting in enhanced fibrinolysis (24). According to European guidelines (25) tranexamic acid, a pharmacologic agent that prevents fibrinolysis, should be administered within 3 hours from the injury and this has been proofed to reduce both the mortality (26) and the need of blood transfusions in trauma patients (27).

## 6.7 Treatment

Apart from the physiological disturbances occurring during massive bleeding there is also a risk that the treatment itself further impairs the blood's ability to coagulate (see Figure 2). In current practice of treating major hemorrhage one should aim for a systolic blood pressure between 80 and 90 mmHg. To achieve this goal, named permissive hypotensive resuscitation, the initial measure is to infuse crystalloids (25). This risks diluting the coagulation factors remaining in the circulation thus impairing the coagulation. Oppositely a restrictive approach, to not fully compensate for the blood volume lost, entails a risk of reducing the tissue perfusion, leading to anaerobic metabolism and acidosis with similarly negative effects on the coagulation.

Figure 2. Hemorrhage and transfusion



As soon as possible the treatment should include blood transfusion in order to replace the lost blood volume and maintain hemoglobin (Hb) at a level that preserves the oxygen delivery to the cells (25). The optimal Hb level and the trigger point for transfusion have been widely debated, taking into



account that the body has several ways to compensate for anemia, such as increasing the cardiac output and increasing the extraction of oxygen from blood to tissue (28). Also the critical point for Hb might differ between individuals and even between organs within an individual (29). The current opinion is that an Hb of 70g/l is well tolerated in otherwise healthy individuals and even lower levels might in the future be proofed safe (30). There are reported cases of patients who refused blood transfusion who survived an Hb value as low as 14g/l (28).

According to European guidelines (25), plasma should be administered in a proportion of at least 1:2 to RBC in massively bleeding patients but should be avoided if the hemorrhage is not significant. The first plasma related factor to reach critically low levels in bleeding is fibrinogen(31). The content of fibrinogen in plasma components is variable (32), and large transfusion volumes may be needed to overcome the initial loss (33). An alternative strategy to infuse plasma, proposed by the same guidelines is to add fibrinogen concentrate to RBC transfusions (25).

Furthermore, the infusion of cold fluids, blood or other, might produce hypothermia which negatively affects the coagulation. For every decrease in degree of Celsius, the coagulation ability is reduced by 10 % (31) . Finally, calcium levels in blood are often reduced during transfusion due to citrate content in blood products which binds free calcium ions. This also has negative effects on the coagulation since calcium is a mandatory cofactor in many processes of the coagulation cascade. Calcium should therefore be kept within normal range during resuscitation (25).

In summary, bleeding is a life-threatening condition that obliges active treatment (34). How this treatment is performed, which components should be used and in what proportions, is a delicate question and might have large influence on the outcome.

## **6.8 The proportion of blood components in transfusion**

When we bleed, we bleed whole blood but when we replace the hemorrhage we transfuse separated blood components; namely RBC, plasma and platelets. A range of studies have showed that how we transfuse might affect the result and there is an ongoing discussion of how the proportions between the different components of transfusion should be to optimize the outcome (35).

In a symposium held in the US in 2005 a new strategy for treatment of massive transfusion was presented. The strategy aimed for a more balanced transfusion where the ratio of plasma to RBC approaches 1:1 (36). This was a major change from previous clinical practice where plasma was administered only when coagulopathy occurred, identified clinically or as deranged coagulation parameters. This previous practice normally resulted in a non-balanced transfusion therapy where the quantity of RBC highly exceeded the plasma quantity, especially early in the time course.

A retrospective review of 10 years' experience from trauma patients in a military setting showed a shift in management over the years with increasing plasma to RBC ratios, decreasing use of crystalloids and introduction of additional treatment with tranexamic acid. During the study period the mortality decreased despite increased levels of injury measured by ISS. The authors' interpretation is that increasing the amount of plasma is beneficial in treating massive hemorrhage (37).

Military settings imply certain forms of injury and certain patient characteristics like younger age and higher proportion of males compared to civilian settings. But even if a range of observational retrospective studies performed in civilian settings also have indicated a survival benefit of increased plasma use (38-44) these results have been criticized for suffering from biases (45). An additional reason to be cautious about interpreting previous result is that civilian settings are considerably different depending on region. For example, penetrating violence is much more common in the US compared to Europe, multi-center studies showing the proportion to be 50% among trauma patients in the former (46) and only 20% in the latter (47). Since different injury mechanisms probably affect the coagulation system in different ways (48), for example ETIC being more common in penetrating compared to blunt violence (49), the benefit from a high plasma ratio might also vary. The possibility that certain treatments have effect in specific settings, but not in others, must be taken into consideration before new transfusion therapies are adopted globally.

To increase the amount of plasma transfusion, without clear evidence of its beneficial effect, has raised concerns since plasma transfusion is associated with higher incidence of acute respiratory distress syndrome (ARDS), multi organ failure (MOF) and other adverse outcomes (50). Others speculate that the increased morbidity, associated with plasma, is the result of more patients surviving and therefore more patients have the possibility to develop adverse outcomes (51).

In 2013, Holcomb et.al presented the first randomized controlled trial (RCT) where they compared high vs low plasma ratio in trauma patients. No significant difference was showed in 24-hour-, 30 day-mortality or in pre-defined adverse outcomes. However, patients receiving high plasma ratio had lower mortality due to exsanguination. Although patients randomized to high ratio received more plasma early in the course, the ratio of plasma to RBC was comparable between the two groups after 24 hours (46), and therefore the comparison of adverse effects might be of limited value. Almost 50% of the included patients were injured due to penetrating violence (46).

## **6.9 Adverse reactions**

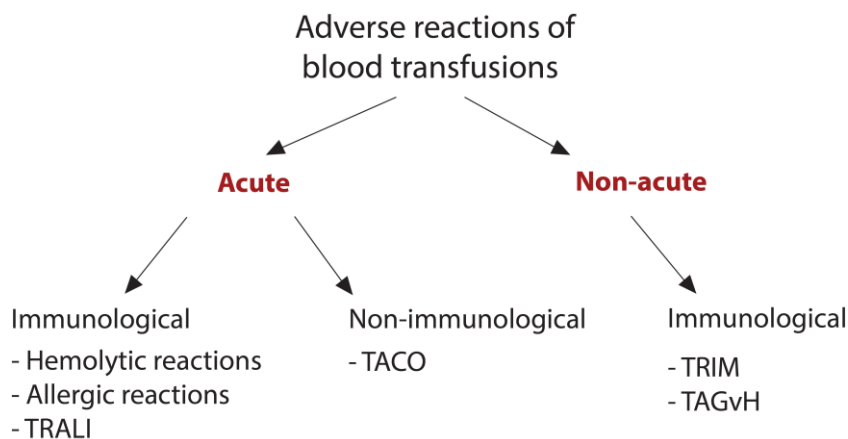
The previously common risk of transmission of infectious disease through transfusion is today under active surveillance and well controlled. Instead, adverse effects due to the immunological event that

a blood transfusion constitutes are of greater concern and likely to create a concrete clinical problem (52).

National hemovigilance protocols which include education, reporting and registration of severe adverse events have been introduced in western countries (53-55). Blood transfusion is today considered a safe treatment and even though an adverse effect appears in 1 of 400 blood transfusions administered (56), only a very small proportion of those events, 1 in 20 000 leads to severe morbidity or mortality (57).

The profile and frequency of adverse events differ between the different component types, hemolytic reactions mainly being associated with RBC, allergic reactions with plasma and bacterial infections with platelets (58). The adverse reactions of transfusion can be divided into acute or prolonged and further divided into immunological or non-immunological (see Figure3). The acute immunological reactions are hemolytic reactions due to non-compatible blood group or the occurrence of irregular antibodies, allergic reactions ranging from urticaria to anaphylactic shock and Transfusion Related Acute Lung Injury (TRALI) which is a non-hydrostatic, immunological pulmonary edema and the leading cause of transfusion related death today (3).

Figure 3.



Among the prolonged immunological reactions are immunomodulation, also called transfusion related immunomodulation (TRIM), which is proposed to suppress the recipient's immunological system (59) and to increase susceptibility to infections (60). Transfusion has, for example, been found to increase risks of recurrence of malignancies (61, 62). Transfusion-associated graft versus host (TAGvH) reactions, although very rare, may also develop after blood transfusion and is characterized by skin manifestations, severe diarrhea, hepatic failure and lead to death in approximately 90% of the cases(3, 63). Immunosuppressed patients or HLA identical relatives are at highest risk of TAGvH reactions. The introduction of leuko-depleted blood products has reduced this risk considerably (57).

The major acute non-immunological reaction is Transfusion Associated Circulatory Overload (TACO). This condition, together with TRALI, constitutes 60% of deaths caused by transfusion (56). TACO is diagnosed by clinical criteria demanding 3 out of 6 symptoms (respiratory distress, evidence of positive fluid balance, increased BNP, radiographic evidence of pulmonary edema, evidence of left-sided heart failure or increased CVP debuting or exacerbating within 6 hours from a transfusion (64). TACO may affect all age groups and known risk factors in the patient are extreme ages and cardiac or renal insufficiency (65). A reduction in the incidence of TACO has been observed after the introduction of leuko-depleted blood components (66, 67) but the condition is still considered to be highly under-reported (68) and carries a high mortality of 50% (69).

Furthermore other, as yet undetermined, adverse effects of blood transfusion have been suggested and explored, such as an increased risk of developing lymphoma (70, 71) or in preterm neonates increased risk of Necrotizing Enterocolitis (NEC) (72, 73). Such associations are however questioned and studies showing no increased risk of lymphoma (74-76) and even protective effect of transfusion on NEC (77, 78) also exist.

A range of observational studies have found associations between blood transfusions and increased morbidity and mortality (79). The risk of developing acute kidney injury (AKI) (80, 81), thromboembolic events (82), postoperative infections (83, 84), ARDS and MOF (50) increases with the numbers of units transfused. A general higher mortality has also been showed in several studies (85-87) and is estimated to increase with 10% for every additional RBC received (85). For obvious reasons clinical trials in bleeding populations, randomizing patients to either transfusion or no transfusion are difficult to perform. However, in RCTs studying thresholds for transfusion, the difference in adverse outcomes between restricted and liberal transfusion strategies has been contradictory (30) and therefore clear causative relationship between blood transfusion and impaired outcome still remains to be proved.

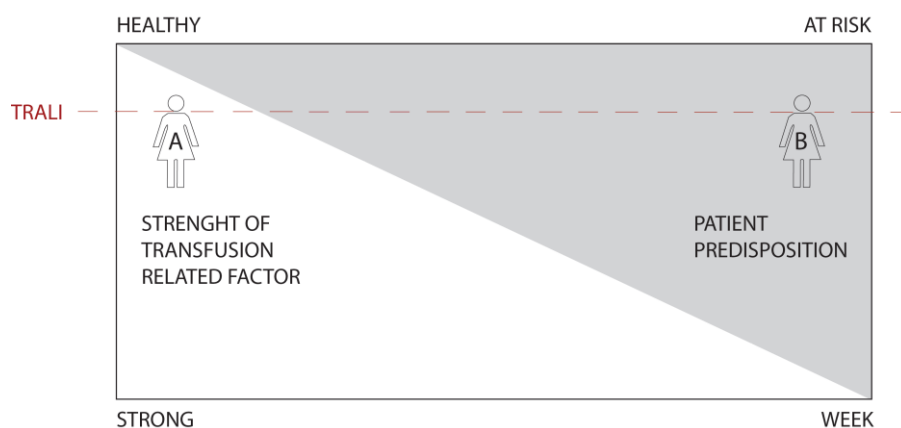
### **6.10 Transfusion Related Acute Lung Injury**

TRALI is a criteria-based diagnosis. In 2004, a consensus conference resulted in the current criteria which are now generally adopted. The criteria are symptoms concordant with ARDS (recent onset of dyspnea,  $\text{paO}_2/\text{FiO}_2 < 300$  mmHg, bilateral effusion on pulmonary X-ray, no evidence for heart failure) that develops within 6 hours from a blood transfusion and where other causes of ARDS are excluded (88). The criteria are seldom fulfilled and have been criticized for not being sensitive enough and miss a large group of patients. The majority of patients who receive large numbers of blood transfusions are severely ill, often with preexisting respiratory insufficiency and they have many different possible causes to develop ARDS. (89). Massive transfusion is also considered an

independent risk factor for ARDS (90), and an overlap between those two diagnoses complicates the reality. As an answer to that criticism, “possible TRALI” has recently been introduced. Possible TRALI allows that other possible causes for ARDS are present, but they should not be as probable as the transfusion administered for causing the disease (91). The true incidence of TRALI is unknown, and the condition is thought to be under-diagnosed and often missed by clinicians, but is estimated to appear in 1 of 5000 to 1 of 12 000 blood transfusions (92). Due to a lack of recognition as well as lack of diagnosis coding in medical records, large-scale observational studies have been difficult to conduct (93). Also there are obvious obstacles in performing clinical trials on critically ill patients with rare and transient conditions and therefore the number of RCTs has been highly limited and mainly focused on the risk of TRALI associated with certain characteristics in blood products (94) or in the processing of blood products (95).

Even though TRALI is considered to be the leading cause of transfusion related mortality, comparatively little is known about the condition. The etiology is thought to be complex and involve factors both in the transfused blood and in the recipient, often presented as a two hit model; patient factors represent the first hit and factors in the transfused blood represent the second hit (93). In a mice model both C-Reactive Protein (CRP) in the recipient (as a marker for patient factor) and antibodies in the blood (as a marker for donor factor) were necessary for the condition to develop (96). Also, the threshold model acts as a way of understanding the development of the condition, where a strong factor in the transfusion is needed in a patient with no predisposition while a weak factor in transfusion might be enough in a predisposed patient (see Figure 4) (97).

Figure 4. Threshold model



The dominating theory about the pathogenesis is activation of primed neutrophils in the recipient through donor derived antibodies towards Human Leucocyte Antigen (HLA) or Human Neutrophil Antigen (HNA) in the transfused plasma. This activation leads to an inflammatory response, mainly in

the lungs, with increased endothelial permeability, leakage of fluid into the interstitium and development of pulmonary edema (93). Many cases of TRALI have been attributed to anti-HLA or anti-HNA antibodies in the donor (98, 99), and since those antibodies have higher prevalence among women, specifically women who have been pregnant (99), the exclusion of female donors from plasma donation has been introduced in many countries (100). Also, using solvent detergent (SD) plasma, pooled from many different donors, with the effect of diluting possibly present antibodies, have been showed to further reduce the incidence of TRALI (101-103).

Still, in 20-50% of the TRALI cases, anti-HLA antibodies are not found (104) and in several cases where an antibody-antigen match has been detected, the patient does not develop the condition (98). Additionally, a not negligible proportion of TRALI cases appear after transfusion of RBC and platelet units, both components with only small volumes of plasma (98). This has motivated alternative theories for factors that can provoke TRALI, for example particles released from erythrocytes and platelets attenuated with storage (105) that in vitro and in animal models have been able to induce the condition (106-109). However, clinical studies on humans have showed conflicting results regarding the risk of TRALI with stored blood products (110). Recently a RCT on 18 human volunteers, mimicking the two-hit-model, showed that patients with induced sepsis and later transfused with autologous RBC stored for 35 days did not develop TRALI or respiratory insufficiency (111). Other factors, related to the donor such as sex, age, smoking and body weight have also been proposed to increase the risk for transfusion related morbidity in the recipient (112) and probably other, still unknown factors in the donor, might play a part in the complex etiology of TRALI.

Patient factors obviously play a crucial role in the risk for TRALI. Among Intensive Care Unit (ICU) patients with gastrointestinal bleeding and comorbid liver failure, the estimated incidence of TRALI has been found to be as high as 30% (113), and in a retrospective review of patients with postpartum hemorrhage 19.7% of the patients were found to fulfill the TRALI criteria (114). Suggested risk factors in the recipient are alcoholism, smoking, hepatic surgery, positive fluid balance and shock (92, 115). Certain patients thereby seem particularly prone to develop the condition and some work has been done to try to predict patients at risk for TRALI in order to specifically reduce risks associated with the transfused blood in those patients (56). Thus, for the moment no validated prediction model for clinical use exists.

### **6.11 Storage time**

Even though certain conditions may or may not be treated with transfusions there are situations where transfusion is unavoidable and at least today, the only available treatment. For these

situations, the focus must be on reducing the associated risks and provide blood transfusion in the safest possible way.

One major concern regarding blood transfusion is the possible detrimental effect of transfusing blood of long storage time. In most Western countries, the current practice is to store RBC for a maximum of 42 days. This practice is based on efficacy and biological changes observed in vitro (116). RBC undergoes a range of changes with increased storage such as decreased levels of 2,3 Diphosphoglycerate (2,3 DPG), accumulation of pro-inflammatory substances (117) and increased deformability in its structure (118). Whether those changes have any impact on the patients receiving the blood is unknown. In 1997 Purdy et.al (119) found increased mortality among septic patients in ICU receiving RBC with long storage time and these results were later confirmed by Koch et.al (120) among transfused patients undergoing cardiac surgery. Since then, many investigations have been published, some suggesting that storage near expiry is associated with adverse outcome (121) while other studies have proposed no clinical relevant effect of longer storage (122, 123). Even though five RCTs recently have showed no risk with increased storage time (124-128), concerns remain, and a possible detrimental effect is not yet excluded. The RCTs have all but one, focused on specific patient groups which limits the generalizability, have been criticized for being under-powered and for comparing storage that does not include the extremes in current range of storage time (129-131).

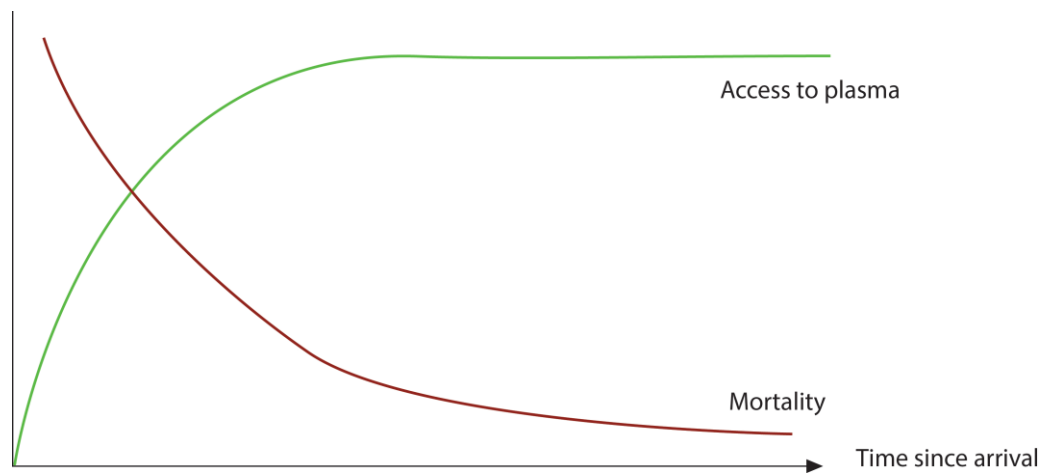
## **6.12 Methodological problems**

The main problem with observational research on blood transfusion is the risk of confounding by indication. Is the blood transfusion the reason for the adverse outcome, or is the adverse outcome the reason for the blood transfusion to be administered? This kind of bias is hard to fully control for in observational designs and publications showing increased mortality, AKI and MOF have all been questioned on this basis (132).

Similarly, the risk for survival bias is highly present, especially when studying different proportions of blood components in transfusion therapy. Many observational studies regarding the optimal ratio of plasma to RBC compare the ratios achieved after 24 hours or during the hospital stay. This has been criticized since the ratio achieved is highly time-dependent due to that, in most circumstances, RBCs are usually available already in the Emergency Room (ER) while plasma in most instances has to be ordered, prepared and delivered from the blood bank before it can be administered. The severely injured patients, with a high probability of dying, will not survive long enough to receive large amounts of plasma and will end up with a low plasma ratio (see Figure 5). This risks biasing the results, and might partly explain the excess mortality showed with low plasma ratio (133, 134).

Others argue for a reversed survival bias where the most severely injured, and who hypothetically would benefit the most from high plasma ratio, are excluded from performed studies due to early death (45).

Figure 5. Survival bias





## **7. Specific aims**

The overall aim of this thesis was to characterize the population of massively transfused patients, study parts of transfusion therapy that might have effect on patient outcome and create a model for identifying and studying TRALI, the major cause of transfusion related mortality. The specific aims were to:

1. Describe the epidemiology of massive transfusion in Sweden and Denmark with regards to indications and diagnoses, transfused volumes, patient characteristics, and patient outcomes.
2. Establish statistical methods for and execute an appropriate analysis of the association between plasma-to-red-cell ratios and risks of death.
3. Investigate the impact of storage time of red blood cells on mortality in transfused patients.
4. Develop and test a statistical method for identifying donors with high risk of causing TRALI in the recipient and further investigate characteristics of those donors.

## **8. Methods and materials**

### **8.1 Data sources**

#### **8.1.1 SCANDAT database**

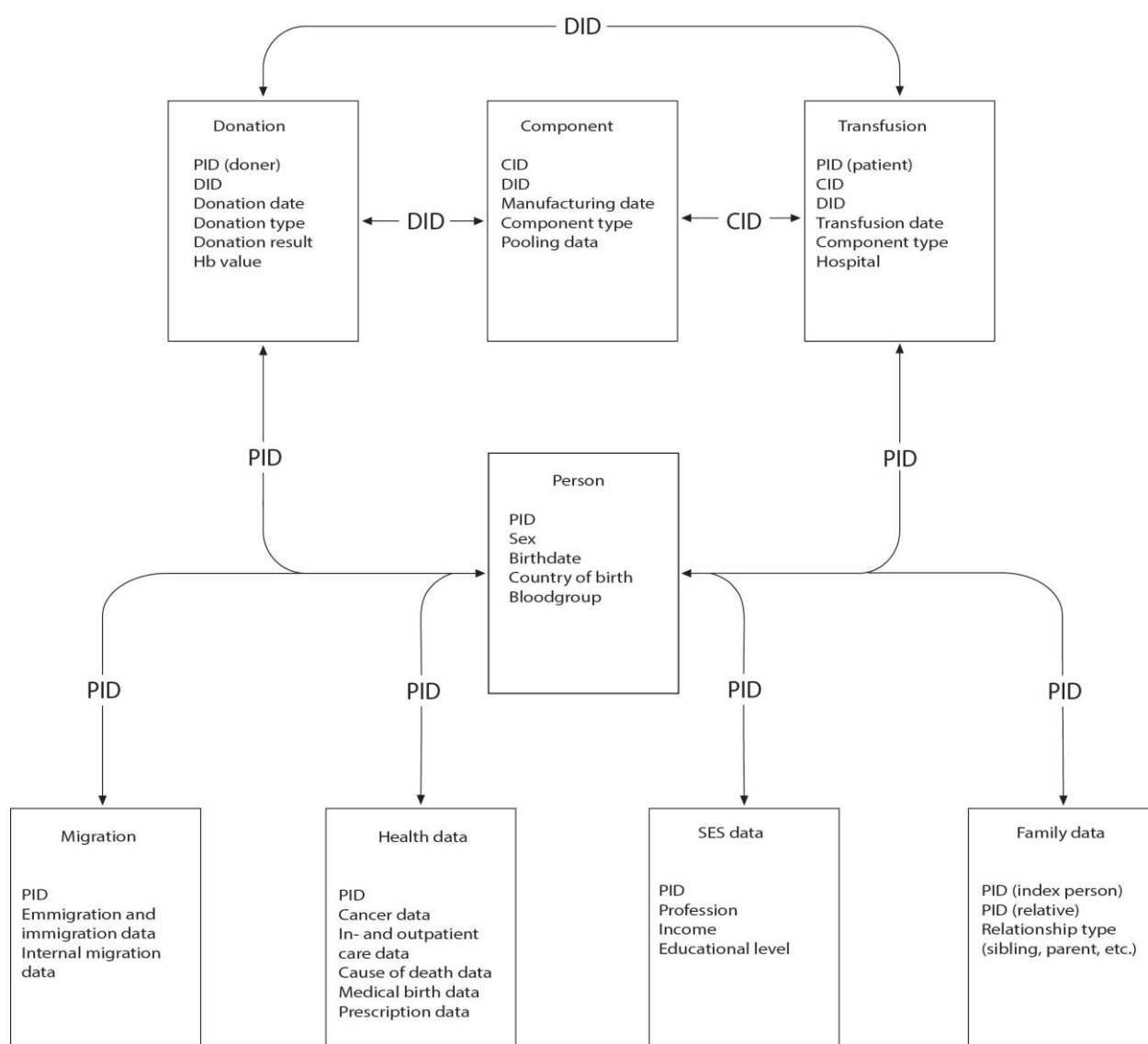
In 3 out of 4 studies included in this thesis, the primary data source has been the Scandinavian donations and transfusions database (SCANDAT2). This database, which collects information on all donations and transfusions in Sweden and Denmark, was originally developed in 2004 (135) and subsequently updated until 2012 (136).

Computerized recording of blood transfusion activity started in Sweden in 1966 and in Denmark in 1981. Although only a few blood centers performed this recording in the beginning it has increased continuously with time and became nearly nationwide in Sweden in 1996 and in Denmark in 1998. Information from the local transfusion registers was gathered, reformatted to fit a common structure and collected in a new database, SCANDAT2. All individuals in the database are possible to identify through a unique national registration number (NRN) that in both Sweden and Denmark is used in all registers as well as in hospital visits (136).

Through the NRN, the data was linked with national population registers to remove inaccurate identification numbers and establish every individual as being alive, deceased or to have emigrated. The linkage also provided information on sex, date of birth, date of immigration and emigration, country of birth, data on migration within the country (i.e., between different administrative regions) and information about first-degree familial relationships within the cohort. Furthermore, linkages were made with national cancer registers, in- and out-patient registers, medical birth registers and cause of death registers. Finally the data was pseudonymized by replacing the NRN with a randomly assigned identification code. A key which enables reidentification of individuals is preserved, in accordance with ethical rules in both countries (136).

SCANDAT2 consists of three main parts; the donation part, the component part and the transfusion part. The donation part contains information about the donor and the donations. The component part contains information about the component such as the manufacturing date and the transfusion part contains information of the recipient and the transfusion date. The three parts are linked by a donation identifier and a component identifier. Through the assigned identification codes both donors and recipients are linked to information from population registers as well as the other registers (see Figure 6) (136).

Figure 6. SCANDAT Database



### 8.1.2 Patient register

Reporting all in-hospital care to the Swedish patient register was initiated in 1987, became mandatory also for specialized and acute out-patient visits from 2001 and has now a full national coverage, except for the primary health care. The register contains dates of admission and discharge as well as diagnosis and interventions according to the International Classification of Disease (ICD) (137). The validity of the register is considered to be high and a review of 900 medical records revealed that the number of false negative cases ranged from 3-5% depending of the diagnosis and

the under-reporting of surgical procedures was 8% (138). The concordance between the patient register and the cause of death register is 99.9% (137).

The Danish national patient register was established in 1977 and has since then expanded to cover all somatic and psychiatric hospital visits in both public and private health care. The register contains administrative data with patient's NRN, type of hospital, date of admission and discharge as well as clinical data about diagnosis and surgical procedures according to ICD (139). The validity of the register has been assessed in a number of studies and its overall quality is found to be satisfactory (140).

### **8.1.3 Regional trauma register**

At Karolinska University Hospital, Stockholm, Sweden a regional trauma register is maintained since 2005 and was subsequently incorporated in the national trauma register (141) in 2011. The hospital constitutes a referral center for all severe trauma cases in the entire region with a catchment area covering around two million inhabitants. All patients admitted to the hospital with a condition that activates the trauma team, as well as patients admitted without trauma team activation but that are found to have ISS > 9 are included in the trauma register. Patients who die after brief resuscitation are also included. Isolated fractures of the upper or lower extremity, drowning, chronic subdural hematoma, burn injury, and hypothermia without concomitant trauma are not included in the registry (142). The register gathers information about ISS, type of violence, primary injury mechanism, Glasgow Coma Scale (GCS) and blood pressure at admission, crucial aspects of interventions and treatments as well as outcome data (143).

### **8.1.4 Medical records**

In study 2 and 4, data collection also included reviews of medical records. The records were retrieved from each hospital's computerized record system or for the earlier study period, a common archive which keeps all paper records. The relevant records were identified through the NRNs and included physician records, nurse records, anesthetic sheets from perioperative care, transfusion records, death certificate as well as results from blood samples, X-rays and other investigations. In the medical records, the exact time for interventions (including transfusions), the time for severe symptoms to appear and the exact time of death were possible to retrieve.

## **8.2 Study Designs**

### **8.2.1 Study 1**

The aim of study 1 was to describe the epidemiology of massive transfusion including incidence, patient characteristics and mortality.

We performed a large-scale descriptive cohort study. The study cohort consisted of all massively transfused patients that were identified in SCANDAT2 between 1987 and 2010 in Sweden and between 1996 and 2010 in Denmark. Massive transfusion was defined as reception of 10 red cell units or more during two consecutive days. This definition is a modification from the usual definition of massive transfusion, i.e. reception of 10 red cell units or more during 24 hours. The modification was done since SCANDAT2 only records at what day a transfusion has taken place and not the exact time, and we wanted to include those who arrive at hospital at late hours and receive 10 transfusions within 24 hours but overlapping two days. However, the modified definition also included patients who received 10 transfusions in a more prolonged way over de facto 48 hours, and those patients may in crucial ways differ from the usual defined population of massive transfusion. Therefore we also performed sensitivity analyses where we defined massive transfusion as receiving 10 red cell units or more within one calendar day. Additionally we identified patients receiving 10 red cell units or more within one transfusion episode, defined as seven consecutive calendar days, and named them non-acute massively transfused. We considered the group of non-acute massively transfused as relevant to include in the study since they are exposed to large volumes of transfusions and by that to the possible risks associated with blood therapy. However, they were described separately since they differ regarding other relevant patient characteristics. Finally we also extracted data on all transfusion episodes during the study time to enable proportion calculations of massive transfusion events to the overall transfusion events.

To establish the indication for massive transfusion we expanded a previous used algorithm (144) where ICD codes from ICD version 9 and 10 were clustered into nine exclusive and hierarchically organized groups. The algorithm was based on a combination of main diagnosis at discharge and codes for surgical interventions made during the hospital stay. For patients with more than one diagnosis, only the diagnosis highest in the hierarchy of the algorithm was considered. This categorization resulted in 9 distinct indication groups; 1) trauma; 2) nontrauma, obstetric care; 3) nontrauma, nonobstetric, cardiac/vascular surgery; 4) nontrauma, nonobstetric, noncardiac/vascular, cancer surgery; 5) nontrauma, nonobstetric, noncardiac/vascular, noncancer surgery, other surgery; 6) other care for hematologic malignancy; 7) care for other malignant disease; 8) other hospital care; or 9) no data available.

### **8.2.2 Study 2**

The aim of study 2 was to assess the association between plasma to RBC ratio and mortality with a method that minimizes the risk of survival bias.

We performed a retrospective cohort study with prospectively collected data. From the regional trauma register we identified all patients admitted to Karolinska trauma center between 2005 and 2010 and who received at least 1 RBC within the first 48 hours. Patients younger than 15 years or older than 90 years were excluded. By using the NRN, the included patients were linked to the local transfusion register where data on number of transfusions, type of transfusions, issue time and blood group of the patient was extracted. The local transfusion register lacks information on the actual time of transfusion administration but we hypothesized it to be close to the issue time (i.e. the time when the blood product is delivered from the blood bank) in this specific patient group where urgent treatment is the default. This was later verified by manually reviewing medical records, anesthetic sheets and transfusion journals from a randomly selected group of patients. Emergency blood units for RBC and plasma are in the study-hospital stored in the ER department, and transfusion of these products is registered in the transfusion register retrospectively with a falsely late issue time. To estimate a correct administration time for emergency products, we used the time between hospital arrival and the subsequent transfusion of a non-emergency unit to each specific patient. This estimation was verified to be correct by manually reviewing medical records, from a randomly selected group of patients, and identifying the actual administration time of emergency units in anesthetic sheets. Finally the exact time of death, for those deceased within 30 days from admission, was retrieved from medical records. By reviewing medical records we categorized all deaths within 30 days into three groups; due to hemorrhage, due to traumatic brain injury or due to other causes.

We compared the risk of death at 7-days and 30-days from admission between patients receiving high versus low plasma to RBC ratio. The cutoff between high and low ratio was set at 0.85, corresponding to the median ratio among all transfused patients in the cohort. We also performed sensitivity analyses where we set the cutoff to 0.75 and 0.66, respectively.

### **8.2.3 Study 3**

The aim of study 3 was to assess the association between storage time of RBC and mortality using three different analytical approaches.

The study was performed as a retrospective cohort study with prospectively collected data. The cohort was identified from SCANDAT2 and consisted of all individuals receiving at least one unit of RBC between 2003 and 2012. Patients younger than 15 years and older than 90 years as well as patients receiving autologous transfusions were excluded. Also excluded were patients with transfusions of unknown storage time. We used three ways of defining storage. Firstly as a discrete categorization of storage time as 1-9 days, 10-19 days, 20-29 days, 30-42 days and a mixed group. Secondly as numbers of old respectively very old blood units received, defined as blood stored for 30

days or more or 35 days or more, respectively, and finally, as mean storage time. Subgroup analyses were performed for each indication group. The indications were retrieved from the patient registers as ICD codes for main diagnosis and surgical interventions at discharge and were then grouped according to the algorithm described in study 1.

#### **8.2.4 Study 4**

The study aim was to test the hypothesis that it is possible, through a statistical model, to identify blood donors who have a high risk of being carriers of some transmissible factor co-responsible for TRALI, and if so, whether this tool can be used to study biological determinants of TRALI.

From SCANDAT2 we identified all blood donors and their transfused patients in Sweden between 1987 and 2012 and in Denmark between 1994 and 2012. We then identified all hospitalization episodes where at least one transfusion was administered and where the recipient was diagnosed with ARDS. Based on this data we derived a score for each donor's risk to contribute to an ARDS diagnosis in the recipient. The risk score was calculated as the difference between the observed and expected number of ARDS cases. We then estimated the association between the highest risk score among all contributing blood donors and the risk of ARDS in the recipient. Based on this analysis, which revealed a markedly increased risk of ARDS in recipients from donors with a risk score  $>2$ , we used a risk-score of  $>2$  as the definition of a high-risk donor.

To establish whether the ARDS diagnosis corresponded to TRALI, we performed a manual review of medical records to assess the temporal association between the transfusion and the development of respiratory distress. This was done by setting up a matched case-case/case-control study where cases were selected among patients with an ARDS diagnosis who had received transfusions from a high-risk donor, control group 1 among patients with ARDS diagnosis who had received transfusion from a low-risk donor (risk score  $< 0$ ) and control group 2 among patients without an ARDS diagnosis but who had received transfusions from a high-risk donor. The aim of the two control groups was to study if receiving blood from high risk donor increased the risk of TRALI, to study possible patient-related factors that contributed to TRALI as well as detecting TRALI cases that were not reported or even missed clinically.

Finally we compared donors with different risk-scores based on descriptive data drawn from the SCANDAT2 database.

## 8.3 Statistical analyses

### 8.3.1 Study 1

Study 1 included descriptive analyses, incidence calculations and survival analysis using the Kaplan Meier method as well as Standardized Mortality Ratios (SMR). The patient characteristics were presented as proportions or median values with interquartile range (IQR). The incidence of massive transfusion was calculated by dividing the number of cases of massive transfusion identified in SCANDAT2, with a modified background population. The background population was retrieved from national population registers and modified by only including habitants in counties covered by SCANDAT2 at that time. The incidence rates were stratified for age, country, calendar period and indication. The short-term mortality was calculated as crude proportion of dead in the cohort, counting 30 days from the day of the last transfusion. In the long-term survival analysis, we used the Kaplan Meier method (145). In our study, individuals were followed from the last day of the first massive transfusion episode until the date of emigration, death or end of follow-up in December 2013, whichever occurred first. The SMR:s were calculated by dividing the observed with the expected number of deaths (146). The expected numbers of deaths were estimated by multiplying the calendar year-, age- and sex-specific follow-up time in the cohort with corresponding stratum-specific mortality rates in the general population. Data of expected deaths was retrieved from the two countries' national population registers.

### 8.3.2 Study 2

In study 2, we used pooled logistic regression to estimate relative risk of death expressed as Hazard Ratios (HR). The majority of previously published papers on plasma to RBC ratios have reported a survival benefit with increasing ratios, but they have been criticized for suffering from survival bias (133, 134). Since we recognized that receiving plasma is strongly time-dependent, we hypothesized that using a time-dependent model, with time since arrival at hospital as time-scale, would be an appropriate way of avoiding biased results while still allowing for inclusion of early deaths. In the study we compared a non-time-dependent model with a time-dependent model to contrast the results using different approaches. A pooled logistic regression model is equivalent to a Cox regression model when the intervals pooled are short (147) and was used for computational reasons. In our study each interval constituted one hour, counting from first transfusion. Follow-up was until death or for a maximum of 7 respectively 30 days. We compared the relative risk of death between patients receiving high versus low plasma to RBC ratio (i.e.  $<85$  or  $\geq 85$ ). Only transfusions administered during the first 48 hours from admission were considered. In the time-dependent model ratios were assessed at the end of each time interval and then pooled to generate a composite estimate, in the non-time-dependent model the last ratio experienced during the 48 hours



was used. Both models were adjusted for time since first transfusion (expressed as a restricted cubic spline with five knots), number of RBC transfusions (expressed as a restricted cubic spline with five knots), sex (male or female), age at presentation (categorized as 15–29, 30–49, 50–74, or 75–90), calendar year of presentation (categorized as 2005–2006, 2007–2008, and 2009–2010), ISS (expressed as a restricted cubic spline with three knots), emergency units transfused (categorized as none, RBCs only, or RBCs and plasma), type of violence (penetrating or blunt), primary injury mechanism (categorized as traffic accidents, falls, assaults, self-inflicted, or other causes), GCS at presentation (3–7 or 8–15), blood pressure at presentation (0–89 or  $\geq 90$ ), and time at first presentation (categorized as 7:00 am to 4:59 pm, 5 pm to 10:59 pm, or 11 pm to 6:59 am).

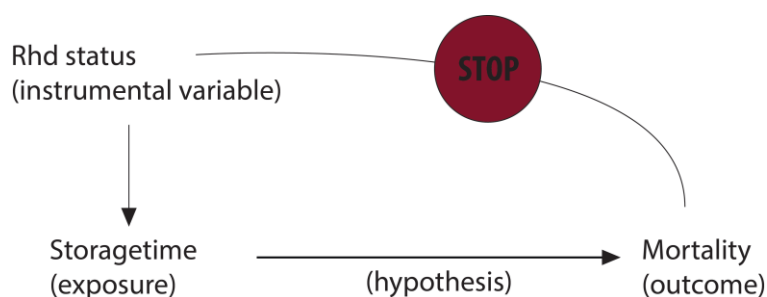
### **8.3.3 Study 3**

In study 3 we investigated the association between storage time of RBC and the risk of death by using Cox regression models and three different approaches of defining storage time. In the first approach we compared the risk of death between discrete exposure groups. Patients receiving numerous blood transfusions and with different storage time were allocated to a group called “mixed category”. Follow-up started at time of the last transfusion in the time-frame of the transfusion episode. The Cox model was adjusted for cumulative number of RBC transfusions (as a restricted cubic spline with 6 knots), ABO-RhD blood group (as a categorical variable), year of first transfusion (as a categorical variable), age (as a restricted cubic spline with 5 knots), sex (as a categorical variable), whether the patient had been transfused previously (as a binary variable), weekday of first transfusion (as a categorical variable), platelets and plasma transfusions (as binary variables), number of days that the patient had been transfused (as a categorical variable) and the indication for transfusion. We used the group receiving blood stored for 10-19 days as reference because this category was the most common and since fresh blood has been proposed to also have detrimental effects on outcome (148).

Although this method assured no overlap regarding the exposure (i.e. storage time), a significant number of patients ended up in the mixed category and we speculated that those patients, receiving larger numbers of transfusions, represented the most severely ill part of the cohort and risked to bias the results. As a way of overcoming this bias, the second approach used a time-dependent model where the exposure was allowed to vary with time. Here, storage time was defined as number of old respectively very old units transfused and were used as a linear term as well as categorized into reception of 0, 1-3, 4-6 and >6 units. This definition also allowed us to estimate a possible association between death and number of blood units stored for long time. Except for introducing the time-varying exposure and starting the follow-up from time for the first transfusion, we used an identical Cox regression model, adjusting for the factors described above.

In the third approach of assessing risk of prolonged storage of RBC, we performed an instrumental variable (IV) analysis. This method is used to overcome the risk of residual confounding (149, 150). An IV is a variable that is associated with the exposure of interest but not independently associated with the outcome (see Figure 7). The stronger association with exposure, the more reliable estimates are produced (151). We used RhD status as the instrumental variable as it is strongly associated with storage time but should have no association with risk of death. The former was verified by highly different mean storage time within the same blood groups but with different RhD status and the latter was confirmed in a supplementary analysis assessing the association between blood group and risk of death in a cohort of all blood donors in SCANDAT2. We performed a Cox regression analysis comparing patients with blood group A+ to patients with blood group A- and patients with blood group O+ to patients with blood group O-. The model was adjusted for hospital, indication and year as these might conceivably confound the association between blood group and mortality. We also performed a two-sample IV-analysis where we first estimated the adjusted association between patient blood group and mean storage time and then in a second step we estimated the adjusted association between blood group and risk of death as a log-linear function. Finally we combined via the inverse variance method results from the stratified A group and O group IV analyses to yield a combined instrumental variable estimate (152).

Figure 7. Instrumental variable



#### 8.3.4 Study 4

In study 4, the risk score was computed as the difference between observed and expected ARDS cases among previous recipient from a particular donor at the time of the donation. We used a logistic regression model to generate the predicted number of ARDS cases. The model included type of donation (i.e. whole blood, plasma, or platelets), calendar year of transfusion (as a restricted cubic spline with 5 evenly placed knots), country (i.e. Sweden or Denmark), age of recipient (as a restricted cubic spline with 5 evenly placed knots), and sex of recipient. The risk score was allowed to change

with every additional donation and was only applicable for the next recipient of each donor. A risk-score above 0 indicates that the disease occurrence of ARDS in previous recipients of that donor was higher than expected. A risk-score below 0 indicates that the occurrence of ARDS was lower than expected. For donors who had not donated previously, the risk-score was set to 0.

In a logistic regression model, we estimated the association between high risk score in the donor and the risk of being diagnosed with ARDS in the recipient. In recipients who received transfusions from more than one donor, the highest risk-score of all donors who contributed to the transfusion episode was used. The model was performed both crude and adjusted for number of transfusions (categorized 1-2, 3-10, 11-30, >30).

For the review of medical records the cases and controls were matched on hospital, date of hospital admission (+/- 2 years), and number of transfusions (categorized as <10, 10-30, >30).

From reviewing medical records, all cases and controls were categorized as non-TRALI, less likely TRALI, possible TRALI, and definite TRALI based on an *a priori* established algorithm. TRALI and possible TRALI in the algorithm corresponded to the generally accepted definition criteria (88) .

Descriptive statistics were used to compare high- and low-risk donors.

## **8.4 Ethical considerations**

All studies included in this thesis were approved by appropriate regional ethic committees and data protection agencies in the two countries.

In general, to keep patient data in registers might by some be perceived as an invasion of privacy. However, this is common practice and mandatory to enable research aiming for improvement of medical care. The SCANDAT2 database does not register any new or otherwise uncollected data but instead gathers them in a way to facilitate epidemiological research. Therefore the database in itself does not further increase the possible problematic issue with invasion of privacy.

All the presented material is at group-level and without any possibility to identify any unique patient who has contributed to the overall results. The data management has been done with de-identified data, except for the hospital record reviews in study 2 and 4. These reviews were done by a single clinician in order to limit the access to sensitive material and the results were immediately de-identified after termination of the reviews. However, that person had access to personal data regarding specific patients. This could of course be seen as a threat to patient privacy since the access did not have any bearing on that specific patient's immediate treatment. On the other hand, data from medical records were necessary in order to answer the hypothesis which had the aim to

improve future treatment strategies for a large group of patients. Our opinion is that the potential benefits outweigh the potential harm and the conduct of the medical reviews was performed with high confidentiality.

The aim of study 4 was partly to identify donors with high risk of causing respiratory complications in the recipients. This aim could be claimed to threaten the positive attitude among blood donors to continue donating blood and participate in studies if they risk being identified as dangerous to patients. On the other hand, blood donors generally aim to do good and identification of some of them as risky for patients would most probably be accepted and seen as important. Further, the possibility to reduce severe adverse reactions in transfused patients is highly beneficial to society and for all individuals who will be exposed to a blood transfusion during their life-time, including several of today's active blood donors.

## 9. Results

### 9.1 Study 1

#### 9.1.1 Study population

We identified 92 057 individuals who experienced a total of 97 972 episodes of massive transfusion. 56 711 episodes were defined as acute and 41 261 episodes were defined as non-acute. Massive transfusion constituted 5.3% of all transfusion episodes during the study period and the massively transfused patients received 13.3% of all transfused blood components. RBC dominated as the blood product administered, followed by plasma and then platelets. The vast majority of massive transfusions were administered to patients who underwent major surgery including cardio-vascular-, cancer- and other surgery. Less than 3 % were massively transfused due to obstetrical reasons. Trauma as an indication for massive transfusion was recorded in 15% of the cases. Among patients with acute massive transfusion more than 50% were 65 years or older. The characteristics of the study population are presented in Table 1.

Table 1. Characteristics of study population.			
	Acute		Non-acute
No. subjects (% of total)	53,836 (58.5)		38,221 (41.5)
Females, N (%)	18,556 (34.5)		14,754 (38.6)
Sweden, N (%)	35,356 (65.7)		22,232 (58.2)
Age at transfusion years, N (%)			
< 18	775 (1.4)		584 (1.5)
18-39	5,569 (10.3)		2,602 (6.8)
40-64	17,166 (31.9)		11,482 (30.0)
65-79	22,82 (42.4)		15,442 (40.4)
80+	7,506 (13.9)		8,111 (21.2)
Median age (IQR)	67 (54-76)		70 (57-78)
Indications, N (%)			
Trauma	8,386 (15.6)		5,788 (15.1)
Obstetric care	1,408 (2.6)		278 (0.7)
Major surgery	36,823 (68.4)		19,574 (51.2)
Cardiac/vascular Surgery	18,725 (34.8)		6,334 (16.6)
Cancer Surgery	5,828 (10.8)		3,405 (8.9)
Other Surgery	12,27 (22.8)		9,835 (25.7)
Hematologic malignancy	676 (1.3)		3,096 (8.1)
Other malignant disease	607 (1.1)		1,269 (3.3)
Other hospital care	5,131 (9.5)		7,585 (19.9)
No data available	805 (1.5)		631 (1.7)
Transfusions per episode, median (IQR)	22 (16-33)		16 (12-22)
Red cells concentrates	13 (11-18)		11 (10-14)
Plasma units	7 (4-13)		3 (0-7)
Platelet concentrates	0 (0-2)		0 (0-1)

### 9.1.2 Incidence

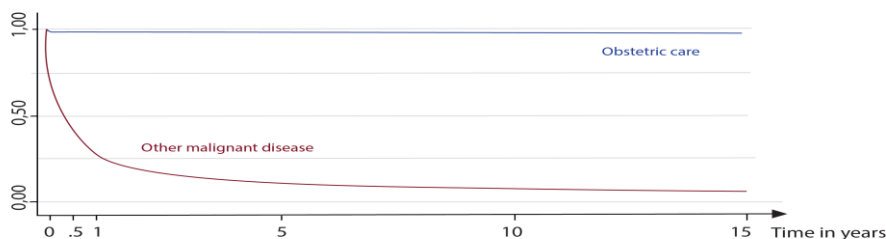
We found differences in incidence, both over time and between countries. In Sweden, the incidence of massive transfusion decreased from 4.2 to 2.4 per 10 000 person years during the study period. In Denmark, the incidence instead increased from 3.9 to 4.5 per 10 000 person years. The incidence was higher in males than in females and peaked in elderly age groups. Regarding indications, the highest incidence was among those transfused for cardiac/vascular surgery.

### 9.1.3 Mortality analyses

The crude 30-day mortality was 24.0%. This proportion differed between indications, being highest among those transfused for “other malignancy” (41.1%) and lowest among those transfused for obstetrical indications (1.0%). Denmark had higher 30-day mortality than Sweden, 27.1% versus 20.8%.

In the long-term survival analysis the median time of follow-up was 3 years, ranging from 0 to 26 years. The 5-year survival was 45.4% overall, decreasing with increasing age. Mortality differed markedly between indication groups, once again being highest among those transfused for “other malignancy” (90.1%) and lowest among those transfused for obstetrical reasons (1.7%) (see Figure 8). Poorer survival was noted among those who received larger numbers of blood products compared to those receiving less.

Figure 8. Survival after massive transfusion



SMRs were considerably higher in the beginning of the follow-up and then decreased successively with time, although not reaching 1.0 even 10 years after the transfusion event. Overall the SMR was 26.2 during the first 6 months of follow-up and 1.6 with 10 years or more of follow-up. SMR was considerably higher for patients receiving > 50 transfusions compared to patients receiving fewer units, but this difference decreased markedly with time.

#### **9.1.4 Other results**

Among those defined as non-acute massively transfused the short-term mortality was lower than for those acute massively transfused, conversely the long-term mortality was higher. The number of repeated transfusion events was also higher in this group. Both the proportion of hematological malignancies and other malignancies were higher in those non-acutely massively transfused and the proportion of obstetric care was markedly lower.

Comparing the two countries revealed similar patterns regarding the majority of variables studied. However patients massively transfused in Denmark had a higher mortality, both assessed by 30-days and by SMR, overall SMR in Sweden was 23.5 (95% CI, 23.2-23.9) and in Denmark 30.9 (95%CI, 30.4-31.5).

Since we used a non-standard definition of massive transfusion, we also performed a sensitivity analysis where we only included those transfused with 10 units or more of RBC within one calendar day (n= 25 039). This group had slightly higher proportions of trauma and obstetric care, consisted of more men and had a higher 30 days mortality 29.3% versus 24.8% compared to the mainly used definition. Regarding other aspects, the sensitivity analysis revealed similar results.

We computed the average plasma to RBC ratio over time. We noted a slight increase in median ratio with time, being highest (0.45) in the recent time-period.

## **9.2 Study 2**

### **9.2.1 Study population**

During the study period we identified 6 124 patients admitted to the regional trauma center. After exclusion of 247 patients who were referred from other hospitals, 5 877 remained for the analysis. The majority of the patients did not receive any transfusions at all, and among the 741 that received a transfusion, 589 received fewer than 10 red blood cells units and 152 received 10 or more units. The median age was 38 among not transfused patients, 44 years among patients not massively transfused and 38 years among the massively transfused. In the three groups the median ISS was 6, 22 and 34, respectively and penetrating violence as the mechanism for injury was 7.7%, 13.9% and 13.2%, respectively. Men constituted more than 70% of the cohort (see Table 2).

<b>Table 2.</b> Characteristics of Study 2 population.			
	No red cell transfusions	1-9 red cell transfusions	≥10 red cell transfusions
No. subjects (% of total)	5135 (87,4)	589 (10,0)	152 (2,6)
Females, N (%)	1377 (26,8)	162 (27,5)	44 (28,9)
Median age at presentation (IQR)	38 (25-53)	44 (31-62)	38 (24-58)
Injury severity score (ISS), N (%)			
0-14	4099 (79,8)	175 (29,7)	12 (7,9)
16-32	923 (18,0)	293 (49,7)	58 (38,2)
≥33	113 (2,2)	121 (20,5)	82 (53,9)
Median ISS (IQR)	6 (2-13)	22 (13-29)	34 (22-44)
Type of violence, N (%)			
Penetrating	396 (7,7)	82 (13,9)	20 (13,2)
Blunt	4739 (92,3)	507 (86,1)	132 (86,8)
Primary injury mechanism, N (%)			
Transport accident	2570 (50,0)	246 (41,8)	77 (50,7)
Falls	1495 (29,1)	164 (27,8)	18 (11,8)
Assault	702 (13,7)	84 (14,3)	16 (10,5)
Self afflicted	176 (3,4)	58 (9,8)	28 (18,4)
Other causes	192 (3,7)	37 (6,3)	13 (8,6)
Glasgow coma scale 8 or below at presentation, N (%)	472 (9,2)	209 (35,5)	75 (49,3)
Blood pressure below 90 at presentation, N (%)	103 (2,0)	78 (13,2)	59 (38,8)
Pre-hospital fluids administered, N	1265 (24,6)	274 (46,5)	105 (69,1)
†Defined as crystalloid, kolloid or hypertonic fluids			

### 9.2.2 Mortality analyses

Overall, 124 (16.7%) of the transfused patients died within 30 days and among those who died, 50% did so within the first 24 hours. The median age among the deceased was 53 years and the median ISS was 34.

In the pooled logistic regression, without taking time into account, the relative risk of death was 2.50 (95% CI, 1.54-4.05) among those receiving low plasma to RBC ratio compared to those receiving high. After introducing a variable for time since arrival into the model, this relative risk decreased to 1.25 and was not longer significant (95% CI, 0.78-2.00). The elevated risk of death noted for older age groups, those with high ISS, those receiving emergency units and those with low GCS or low blood pressure at admission, were similar in both analyses.



### **9.2.3 Other results**

Among the patients included in the study, and where International Normalized Ratio (INR) at admission was available (n=221), we found that almost 20% had deranged values ( $>1.2$ ) at arrival. This number was highest (31%) among those massively transfused. 70% of the massively transfused patients had been resuscitated with crystalloid and /or colloid fluids before admission, i.e. in the pre-hospital setting.

The causes of death were distributed as follows; hemorrhage 38%, traumatic brain injuries 43.5% and other causes of death 18.5%. When assessing mortality we also investigated late mortality, defined as more than 7 days from admission. This late mortality was highest (24%) among the group receiving 4-9 units of red blood cells and not among those massively transfused.

We tested for possible interactions between the effect of plasma ratio to amount of units transfused and ISS. We could not find any variation of the effect based on number of RBCs ( $p=0.46$ ) nor based on ISS ( $p=0.89$ ).

In the sensitivity analyses with different cut-offs defining high and low plasma ratio, the results remained the same, with a significant trend towards higher mortality with low plasma ratio in the non-time-dependent analysis and no significant association in the time-dependent analysis.

Over the study period we noted an improved survival overall. The relative risk of death was 2.3 (95%CI, 1.21-4.51) 2005-2006 compared to 2009-2010. The time from admission to first transfusion, as well as the plasma ratio remained stable over time. However, the time from admission to transfusion of the first plasma unit was markedly reduced from median of 2.57 hours 2005-2006 to a median of 1.35 hours 2009-2010.

## **9.3 Study 3**

### **9.3.1 Study population**

Of 972 547 patients transfused during the study period, we excluded 85 655 younger than 15 years or older than 90 years, 543 who received autologous transfusion and 31 487 who received transfusion of unknown storage time. 854 862 patients remained for the subsequent analyses. The majority of patients were female (56.2%) and median age was 72 years. Age, sex, and indication for transfusion were nearly equally distributed among the categories of storage time. The median number of RBC transfused was 2 among all categories, except for the mixed category where median number of units was 4. The ranges of number of units transfused, varied considerably, being narrowest (1-37) in the category where storage time was 30-42 days and widest (2-212) in the mixed category. For further details see Table 3.

Table 3. Characteristics of Study 3 population.					
	Storage time of RBC				
	0-9 days	10-19 days	20-29 days	30-42 days	Mixed age
Overall, N (% of total)	190 245 (22.3)	278 207 (32.5)	113 481 (13.3)	46 474 (5.4)	226 455 (26.5)
Sweden	107 339 (56.4)	163 879 (58.9)	68 757 (60.6)	35 673 (76.8)	127 977 (56.5)
Denmark	82 906 (43.6)	114 328 (41.1)	44 724 (39.4)	10 801 (23.2)	98 478 (43.5)
Female sex, N (%)	109 474 (57.5)	160 769 (57.8)	65 731 (57.9)	26 740 (57.5)	117 974 (52.1)
Age, median (interquartile range)	71.2 (58.4-80.4)	72.5 (60.3-81.2)	72.9 (61.2-81.2)	73.2 (61.4-81.5)	71.8 (59.3-80.6)
Blood group, N (%)					
A+	81 896 (43.1)	103 262 (37.1)	27 831 (24.5)	7 420 (16.0)	65 045 (28.7)
A-	4 286 (2.3)	12 547(4.5)	14 176 (12.5)	6 374 (13.7)	16 526 (7.3)
AB+	4 580 (2.4)	5 030 (1.8)	4 213 (3.7)	5 100 (11.0)	10 842 (4.8)
AB-	483 (0.3)	752 (0.3)	794 (0.7)	757 (1.6)	2 719 (1.2)
B+	15 911 (8.4)	16 365 (5.9)	8 804 (7.7)	4 938 (10.6)	20 845 (9.2)
B-	1 359 (0.7)	1 880 (0.7)	2 120 (1.9)	1 569 (3.4)	5 299 (2.3)
O+	50 769 (26.7)	98 799 (35.5)	31 586 (27.8)	7 824 (16.8)	60 887 (26.9)
O-	2 646 (1.4)	7 867 (2.8)	11 742(10.4)	8 465 (18.2)	16 088 (7.1)
Unknown blood group	28 315 (14.9)	31 806 (11.4)	12 215 (10.8)	4 027 (8.7)	28 204 (12.5)
Number of RBC transfusions, median (range)	2 (1-79)	2 (1-69)	2 (1-53)	2 (1-37)	4 (2-212)
Previous transfusion, N (%)	33 951 (17.9)	49 801 (17.9)	20 549 (18.1)	9 277 (20.0)	42 384 (18.7)
Identical ABO-Rh transfusion, N (%)	155 742 (81.9)	237 715 (85.5)	97 050 (85.5)	39 936 (85.9)	174 739 (77.2)

### 9.3.2 Mortality analyses

In the discrete exposure group analysis, we found no significant difference in risk of death between the different categories of storage time, neither with 30-days nor with 1-year follow-up. The relative risk of death was 0.99 (95% CI, 0.95-1.02) for those receiving blood stored 30-42 days compared with those receiving blood stored 10-19 days. This corresponded to an adjusted cumulative mortality difference of -0.2% (95% CI; -0.5%—0.1%). Also, we did not find any mortality differences when we stratified for indications.

In the time-dependent model we could not find any significant association between increasing numbers of old units received and mortality (HR 1.00; 95% CI, 0.99-1.01). When comparing patients transfused with > 6 units stored for 35 days or more with patients not receiving any very old units, the HR was 0.98 (95% CI, 0.90-1.08). Equally we did not find any increased risk of death among patients transfused with more than 6 units of fresh RBC (stored < 7 days) HR 1.00 (95% CI, 0.95-1.04) compared to patients not transfused with any fresh RBC.

With the instrumental variable analysis, we found no association between RhD status and the risk of death, HR 1.00 (95% CI, 0.97-1.03) comparing A+ to A- and 1.01 (95%CI, 0.98-1.04) comparing O+ to O-. The combined two-sample instrumental variable analysis did equally not reveal any association between mean storage time and risk of death, HR 1.01 (95% CI, 0.99-1.03).

### **9.3.3 Other results**

As we hypothesized that the risk of changing exposure group was associated with baseline exposure, this was assessed in a logistic regression analysis. The risk of changing exposure group differed between the exposure groups at baseline and was 1.41 (95% CI, 1.35-1.47) for those originally receiving blood stored 30-42 days compared to those originally receiving blood stored 10-19 days.

Since the instrumental variable analysis is only valid if the instrumental variable in itself has no independent association with outcome, we performed an analysis where we studied the risk of death comparing different blood groups. This analysis was made among healthy donors identified in SCANDAT2 between 1968 and 2012. We included 1 601 342 donors and followed them for a total of 26 517 461 person-years. 79 432 deaths occurred during the follow-up. We found no association between RhD status and mortality, HR 1.01 (95% CI, 0.99-1.03) for donors with RhD- compared to donors with RhD+. This lack of association persisted when stratified for blood group A and O.

## **9.4 Study 4**

### **9.4.1 Study population**

We identified 1 758 916 patients with a total number of 3 045 770 hospitalizations where the patient received at least one transfusion. Among all hospitalizations, 3 570 resulted in an ARDS diagnosis at discharge. We found no variation in ARDS diagnosis over time. There were a total of 1 189 182 donors in the cohort. Of those 86 543 had donated a blood product to a recipient who subsequently was diagnosed with ARDS. Among all donors, the median risk score was -0.04 with a range from -3.75 to 7.93.

Patients with ARDS were younger than patients without ARDS, median age 63 (IQR, 49-71) versus 73 (IQR; 59-82). They received considerably higher numbers of transfusions and among all transfusions, more plasma components. However, among patients with ARDS as many as 31% had not received any plasma at all. The proportion of women was less among ARDS patients compared to patients without ARDS, 38.6% compared to 54.2%. Patients with ARDS had higher 30-day mortality, 34.3% than patients without ARDS, 10.2%.

### **9.4.2 Association of risk score and the risk of ARDS**

We found a strong association between the maximum risk score of the donor and the recipient's risk of being diagnosed with ARDS, Odds Ratio (OR) 34 (95% CI, 28-42) when comparing patients who received blood from a donor with a risk-score of >4 compared with patients receiving blood from a low risk donor (risk score <0). After adjusting for number of transfusions, the OR markedly decreased but still indicated a higher risk of death, OR 3.4 (95% CI, 2.7-4.3). We also performed analyses restricted to donors with less than 20 donations, showing even higher associations between risk-

score in the donor and ARDS in the recipient; OR 47 (95% CI, 29-77) for unadjusted model and 5.4 (95% CI, 3.2-8.9) for the adjusted model.

### 9.4.3 Medical review of cases and controls

294 patients were matched and randomly selected for the hospital record review. They were distributed as 127 cases (i.e. ARDS diagnosis and transfusion from donor with risk score >2), 44 control group 1 (i.e. ARDS diagnosis and transfusion from donor with risk score <0) and 123 control group 2 (i.e. no ARDS diagnosis and transfusion from donor with risk score >2). In 19% of the patients a proper categorization was not possible to perform due to lack of relevant medical records and/or investigation results. The median DES did not differ between patients we were able or unable to categorize. In the remaining 239 patients, we found only one definitive TRALI case, which belonged to the control group 1. Additionally 20 patients were categorized as possible (7) or less likely (13) TRALI (see Table 4). No suspicion of TRALI or a transfusion reaction was raised in the medical records for any of these 21 patients.

Table 4. Cases and controls.			
	Cases	Control group 1	Control group 2
Total, N	127	44	123
TRALI, N	-	1	-
Possible TRALI, N (%)*	3 (2)	1 (2)	3 (2)
Less likely TRALI, N (%)*	6 (5)	4 (9)	3 (2)
No TRALI, N (%)*	89 (70)	30 (68)	99 (80)
Not possible to categorize, N (%)*	29 (23)	8 (18)	18 (15)
Maximal risk score, median (range)	2.8 (2.0-7.3)	-0.2 (-0.3--0.0)	2.8 (2.0-7.4)
Female, n (%)	36 (28)	16 (36)	53 (43)
* Numbers may not adopt due to rounding			

### 9.4.4 Other analyses

In descriptive analyses comparing donors with high- and low-risk score (see Table 5) we found differences in the total number of donations which were significantly higher in high-risk donors compared to low-risk donors, median 73 donations (IQR, 56-92) among donors with risk-score >4 vs 7 donations (IQR, 3-16) among donors with risk-score <0. The proportion of women as well as the proportion of donors with blood group B decreased with increasing risk-score. Oppositely the proportion of donors with blood group AB and the proportion of donors with a history of previous pregnancies were highest among donors with high risk-score.

Table 5. Characteristics of donors stratified by risk score				
	DES <0	DES 0-2	DES 2.1-4	DES >4
Number of donors (%)	1 088 013 (91.5)	98 375 (8.3)	2 659 (0.2)	135 (0.0)
Median age at first donation (IQR)	33.2 (24.6-43.7)	34.8 (26.2-44.0)	37.0 (28.9-44.7)	38.4 (29.4-46.3)
Median number of donations (IQR)	7 (3-16)	25 (13-41)	52 (37-69)	73 (56-92)
Median number of ARDS cases (range)	0 (0-6)	1 (1-6)	3 (3-15)	5 (5-11)
Female (%)	532 867 (49.0)	37 965 (38.6)	613 (23.1)	27 (20.0)
Blood group				
A	468 929 (43.4)	43 650 (44.4)	1 245 (46.9)	61 (45.2)
B	121 776 (11.2)	7 709 (7.8)	103 (3.8)	5 (4.0)
AB	54 232 (5.0)	5 333 (5.4)	208 (7.8)	17 (12.6)
O	443 385 (39.8)	41 359 (42.0)	1 101 (41.4)	52 (38.5)
Previous pregnancy (%) *	66446 (20.8)	7 167 (30.0)	167 (47.0)	10 (47.6)
Previous transfusions (%)	20 677 (1.9)	1 830 (1.9)	55 (2.1)	6 (4.4)
Ever diagnosed with ARDS	44 (0.0)	7 (0.1)	0 (n.e)	0 (n.e)
* In female donors				

To verify that the number of donors with risk score >2 was greater than would be expected by chance alone, we performed a simulation analysis in the actual data set where we randomly distributed the ARDS cases among the donors. The simulation analysis resulted in 1347 donors (95% CI, 1277-1427) with risk score >2. This should be compared to the markedly higher number of donors with risk score >2, 2659, that were identified in the original analysis.

## **10. Methodological considerations**

### **10.1 Observational vs randomized studies**

When studying both the effect of plasma to RBC ratios and the impact of storage time on survival, we are likely looking for small treatment effects that may still have a considerable clinical significance. The possibility to detect small differences requires both large population samples and a reasonable spread in the exposure. Such circumstances are hard to achieve in RCTs. Well conducted and large-scale observational studies might therefore be an attractive alternative (153, 154).

Although RCTs are considered to be the gold standard when assessing causality or when studying treatment effects, and if well performed, creates estimates with high internal validity, the method have certain disadvantages (155). Among those are the difficulty to enroll sufficient number of patients to ensure a large sample size, both because of costs, the ambition to keep homogeneity and the lack of time (156). This might restrict the external validity and limit the possibility to generalize to broader populations (157).

SCANDAT2 is a database with almost full coverage of all transfusions in two countries, the data is collected and registered independently and prior to origin of research questions and the linkage to other national population register provides robust and reliable data that enables valid results. The conditions for detecting small differences in outcome are optimal. On the other hand, in observational studies the main limitation is the possibility that allocation of exposure is somehow related to prognosis, i.e. confounding by indication, and even when carefully adjusting for possible confounders, the risk of residual confounding always exists (158, 159).

The studies included in this thesis were designed to minimize the risk of confounding in ways discussed below. Where possible we created models adjusted for recognized and potential confounders and where possible we compared different analyses methods in order to produce reliable and non-biased results. As a consequence, our methods might not be immediately intuitive and our statistical approaches included certain assumptions that we have strong reasons to believe are fulfilled but that cannot in every situation be proven. It is therefore reassuring that our results from both study 2 and particularly study 3 are in line with results from several randomized controlled trials (46, 124-128).

A combination of data from well conducted large observational studies, as ours, and from RCTs, indicating the same result, assure both the detection of small yet clinically significant effects as well as good control for factors other than the exposure of interest. In combination, results from RCTs and observational studies can be considered to have both high internal and external validity (156, 160).

## 10.2 Confounding by indication

Confounding by indication, meaning that a patient receives a certain treatment, not by chance, but due to certain factors that are associated with the outcome of interest, is one of the biggest concerns in observational studies (161). In study 1 we compared the risk of death among massively transfused patients with a standardized background population. The markedly increased SMR in the group massively transfused, showed in study 1, is not an indication of a causal relationship between blood transfusions and risk of death, but instead the expected result of confounding by indication.

Massively transfused patients are much sicker and much more prone to die than the general population. Massive transfusion is the consequence of the disease or injury rather than the cause of it.

In study 2, confounding by indication would arise if the clinicians are prone to transfuse more plasma in the most severely injured patients compared to those less injured. This would result in the more severely injured being located into the group receiving high plasma to RBC ratio, showing higher mortality in this group and confounding the results. Our results showed no difference in mortality between high or low plasma ratio and oppositely a higher mortality in the low plasma ratio group in the non- time- adjusted model. If confounding by indication was present, our study implies that high plasma ratio is even more beneficial than the estimates indicates, since despite more severely injured in high plasma ratio group, the mortality was still lower. Confounding by indication could also theoretically take the other direction if clinicians transfuse less plasma to the most severely ill patients. However, this seem contra intuitive from a clinical perspective and the only situations where such a circumstance would appear, is if the patients die before having time to receive plasma transfusion. This would instead be considered a survival bias, where the patient has to survive long enough to be able to receive a certain treatment. Likely this is the explanation for the difference in mortality risk seen between our two models and is further discussed below.

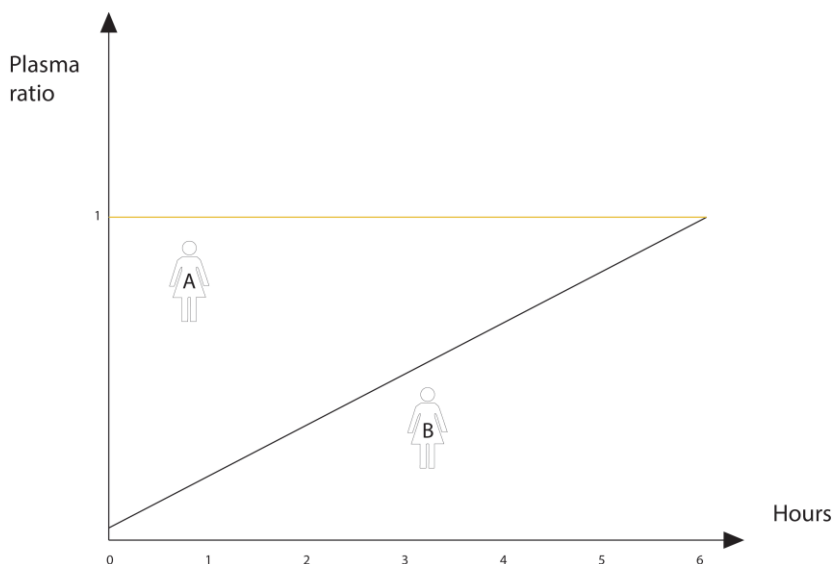
Regarding study 3, there are known circumstances where the clinicians order blood units with specific characteristics, which in turn could affect also the storage of these units. These situations include ordering blood to patients with known erythrocytes antibodies, neonates and chronically transfused patients. Such patients could therefore receive blood units of shorter storage time than usual (162). This is rare and in a large study, such as ours, would have minimal, if any, effect on the results. However this could be a partial explanation for the observed increased risk with transfusion of fresh blood showed in other studies (148), if specific patient groups with higher mortality are more prone to be transfused with fresh blood.

### 10.3 Survival bias

Survival bias arises when patients who live longer have higher probability to receive a certain treatment. If only patients that survive are included in the study it introduces a selection bias that can falsely show a beneficial effect of the treatment (163). The critical point is when the inclusion criteria are fulfilled and when mortality is assessed. In studies where early mortality is assessed this bias might be more pronounced (164).

In trauma patients early mortality is a fact, around 42% of civilian trauma patients die within one hour from admission (12) and in military trauma as many as 78% are dead within the same time frame (165). Survival bias has therefore been the major criticism when discussing results from observational studies showing beneficial effect of raising plasma to RBC ratio (133, 134). How long a patient survives is both highly associated with the severity of the original injury and with the patient's possibility to receive plasma and achieve a high plasma ratio. If excluding early deaths in order to prevent this bias, a possible beneficial effect of high plasma ratio could be hidden. Our study was specifically designed with the aim to overcome these problems. With our time-dependent model, the amount of plasma was compared at each 1-hour-interval from admission throughout the first 48 hours (see Figure 9) and thereby we covered also early deaths and took the possible prolongation of administering plasma into account.

Figure 9. Time-dependent model



### 10.4 Number of transfusions

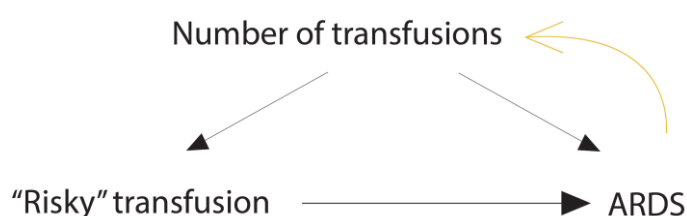
Except for being the most important confounder in observational transfusion research (166), number of transfusions, even when adjusted for, might lead to residual confounding if the adjustment is not accurate. The probability to receive a transfusion with a rare characteristic, in this thesis blood stored



for a long time or blood with risk to initiate TRALI, increases with every additional unit of transfusion received. Thereby, the most severely ill patient receives the highest number of transfusions and will have the highest probability to receive a transfusion with a rare characteristic. The association between number of transfusions and risk of death is not linear and simulations with different ways of adjusting for number of transfusions has showed that this is best taken care of by using cubic spline (167). Cubic splines allow for flexible forms of the relationship between a continuous variables and outcome (168). We used this method in both study 2 and 3 and think we thereby handled this important confounder in the most appropriate way.

In study 4, number of transfusions is of course a confounder since it indicates a more severely ill patient, with an inherent higher risk of developing ARDS irrespective of transfusion. However the outcome of interest, namely ARDS, might further lead to more transfusions and thereby we might incorrectly adjust for a variable that is also affected by outcome (see Figure 10) (169). Since we have no possibility to determine whether a transfusion is administered before or after the ARDS diagnosis, number of transfusions should not be dealt with using standard methods for confounding (170). After adjusting for number of transfusions the estimates were reduced but still a significant association between the risk-score of the donor and risk of developing ARDS in the recipient existed. However, we advocate caution in interpreting the adjusted estimates since inappropriate adjustment might in fact introduce a bias.

Figure 10.



## 10.5 Blood allocation

Blood allocation, i.e., how a certain blood component reaches the recipient, has previously been thought of as a random process. When the clinician orders blood, the blood bank gives out a component based on blood group but otherwise due to a “first in, first out” principle and possible risk factors attributable to the blood component would therefore be equally or at least randomly distributed among transfused patients. The blood bank is blinded to the patient’s condition and the clinician is blinded to certain factors in the blood component, and thereby the process can be

considered as a naturally double-blinded randomization (171). Also, blood allocation has been showed to mainly be based on administrative factors and not on patient characteristics (172) and since the administrative factors may vary according to local contexts, adjusting for hospital might still be necessary.

In study 3 we show that even if the allocation is random or based on administrative issues, it is of great importance to still adjust for possible confounders since, storage time in this case, will be unequally distributed at least based on number of transfusions and blood group. This unequal distribution, leading to differences in mean storage time between blood groups, enabled us to use an Instrumental variable approach.

In study 4 one possible explanation for the association between risk score in the donor and ARDS in the recipient could be that blood units from certain donors were allocated more frequently to patients at risk of developing ARDS. This could be the case if blood from donors with specific characteristics would be transfused more commonly to critically ill patients. Since AB plasma is the universal plasma and can be used for all recipients irrespective of blood group, it is used preferentially in emergency situations. Blood from donors with blood group AB could thereby be allocated more frequently to critically ill patients. However, even if our data showed that donors with blood group AB were more frequent in high risk score- compared to low risk score-groups, the differences were relatively small and not in the magnitude to explain the results.

## **10.6 Misclassification**

Misclassification is a form of information bias due to errors in measurement of exposure, outcome or confounding factors. Misclassification can be non-differential where all patients in the study have the same probability to be misclassified or differential where the probability of being misclassified is related to the status of the patient (i.e. exposed/not exposed or having/not having the outcome of interest). Differential misclassification is often considered to be the most serious form of misclassification since it can bias the results in either direction compared to non-differential misclassification that tends to bias the result towards null (173-175). However, reporting falsely null results due to non-differential misclassification can also be detrimental.

All studies included in this thesis were based on exposure data that was collected prospectively, independently of the research hypothesis as well as of the outcome. We have, for example, no reason to believe that the ratio of plasma to RBC or the storage time of RBC would be detected differently between survivors and non-survivors. Therefore we consider the risk of differential misclassification to be very small. We also consider the risk of non-differential misclassification to be low, since recording of blood transfusion is mandatory in both countries and both type of

component, number of transfusions and storage time are highly concrete and well defined variables. It seems improbable that the null results presented in this thesis would be a consequence of non-differential misclassification.

Misclassification of outcome is even more improbable since data of deaths were extracted from each national death register, having full coverage and extremely high validity.

The statistical analyses used in Study 4 are based on a risk-score. We developed the risk-score as a difference between observed and expected cases of ARDS, in order to be able to compare risks in donors with highly different number of donations. However, stratified analyses showed higher association between the risk score of donors with less than 20 donations and the risk of ARDS in the recipient. This indicates that the risk-score is a stronger signal of a risky donor in those with few numbers of donations. Our inability to detect any true TRALI cases among recipients from high risk donors might partly be explained by the risk-score not being discriminatory enough and that certain donors were misclassified as high risk without actually carrying such a risk factor.

Also, the algorithm of generating donor risk score is entirely based on the number of recorded ARDS diagnoses among prior recipients of each donor. The accuracy of the algorithm is thereby dependent on a uniform way to register ARDS, both over time, between hospitals and on the completeness of such registrations. The accuracy in ARDS diagnosis, both clinically and in medical records, has been shown to be limited (176) and many ARDS cases remain under-recognized by clinicians (177) and this could additionally have influenced our results.

## **10.7 Classification of indications**

In both study 1 and 3 we present results stratified by indication. The classification of indication was based on the main diagnosis at discharge as well as surgical procedure codes according to International Classification of Diagnosis (ICD). The use of ICD to determine the cause of bleeding and thereby the indication for transfusion, has been proven adequate by McQuilten et.al. (178). In paper 1, the study period covered the change from using ICD version 9 to usage of ICD version 10. Both differences in practice between countries and hospitals and differences arising from the conversion from one version to another, introduce some uncertainty regarding the recorded diagnosis (179). However, we used a broad categorization with the purpose to create a robust algorithm not threatened by local or time-dependent differences. The lack of more detailed information of indication is a consequence of this approach.

## 10.8 Observer bias

Observer bias is a specific form of misclassification where the observer who is aware of the exposure status of an individual may be consciously or subconsciously prone to assess outcome according to the study hypothesis (180). In study 4 the identification of outcome, in this case the fulfilling of TRALI criteria, was made after the categorization of exposure. A risk of observer bias, where the person reviewing the records is more prone to detect TRALI in patients known to be exposed to a high risk donor is thus realistic. To avoid this, we ascertained that the person who reviewed the medical records was at that moment blinded to the exposure status of each patient. If an observer bias would be present it would increase the number of TRALI cases in the exposed group, compared to the true incidence. We found less TRALI cases than hypothesized and therefore we see no risk that observer bias might have influenced the result.

## 10.9 Defining cohorts

Defining the cohort to be studied is of great importance when assessing the validity of the obtained results. Both study 1 and 2 were designed to answer questions about patients massively transfused. Even though a world-wide used definition of massive transfusion exists, i.e. receipt of 10 units of red blood cells or more within 24 hours, this definition is associated with certain difficulties. The definition is retrospective in its nature, meaning that a patient is only known to be massively transfused after fulfilling the criteria, and cannot be identified prospectively. Also, patients who die early and who are not alive long enough to receive 10 units of RBC, will not be included in studies on a massively transfused population (181).

In study 1 our definition resulted in an unavoidable exclusion of those severely injured with major hemorrhage, but who did not survive long enough to be able to receive 10 units of RBC. If improvement in care of those with major hemorrhage has taken place during the study period, this would result in more individuals surviving long enough to fulfill the criteria, and consequently the incidence of massive transfusion would increase. In Sweden we instead saw a decrease in the incidence of massive transfusion over calendar years, which were equally distributed among the different indications and not attributable to a concurrent reduction in cardiovascular surgery activities.

In study 2 all patients who received at least 1 unit of RBC were included in the analyses and the risk of mortality was assessed at each hour from the reception of the first blood transfusion. In this study also early deaths were included and possible beneficial effects of high plasma ratio, which theoretically could be most pronounced in the group of most severely injured, had a chance to be

detected. However, study 2 included a small number of patients and did not allow for subgroup analyses and was not powered to detect small but yet clinically significant effects.

### **10.10 Missing data**

In the case-case/case-control part of study 4, we were unable to categorize 19% of the patients due to insufficient information or missing medical records. These patients were to a certain degree unequally distributed among the cases and controls and the majority was patients with hospitalizations early in the study period with non-complete files in the archive. If all patients with missing data were actually TRALI cases we would have had additionally 55 cases in our data changing our results in a remarkable way. However, we see no reason to believe that is the case. On the contrary, a patient with severe respiratory symptoms is preferably treated at higher level of care where investigations and documentation is more frequent and missing data would thus be less common among these patients. Our inability to detect true TRALI cases should therefore not be attributed to missing data.

### **10.11 Residual confounding**

Residual confounding is the bias that remains after controlling for confounding in the design, implementation and analysis of a study (182). Essentially all observational studies suffer some degree of residual confounding. Factors that are unknown to confound the association studied, factors inadequately measured or factors that we were unable to control for might have a residual confounding effect on the estimates and can both increase or decrease the true association (183).

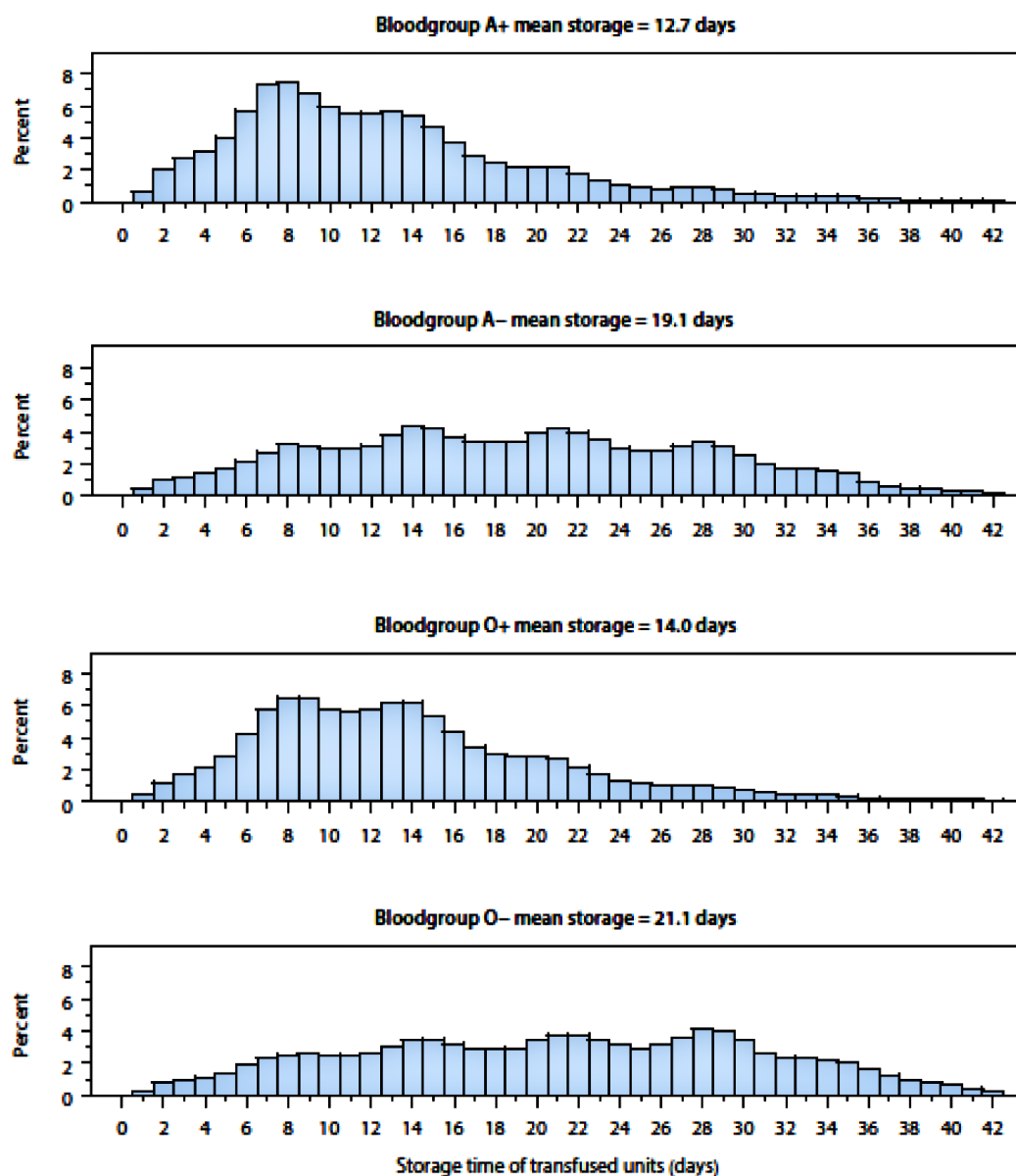
In study 1 we did not draw any causative conclusions and the results are strictly descriptive in nature which makes the discussion of residual confounding irrelevant.

In study 2 we controlled for a range of factors that could possibly affect the association. A proper categorization of study variables might be challenging in trauma research and adjusting for imperfectly defined variables might lead to residual confounding. Especially the time-factor is important to define and categorize in sufficiently short intervals to allow for difference in transfusion patterns among the patients (184). We cannot guarantee a total lack of residual confounding but our data is based on reliable registers with accurate information, which enabled us to define and categorize each variable in a detailed way. We also believe our time-interval of one hour to be adequately narrow to capture the different transfusion patterns present.

Instrumental variable analysis is a suggested method for producing results less affected by residual confounding. The instrumental variable is used as a proxy for the exposure variable when studying its association with outcome (149, 150). In study 3 we used blood group as the instrumental variable. A

patient's RhD blood group does not have any known association with mortality but is strongly associated with the storage time of RBC received. Unknown factors that affect the length of storage of the transfused unit or known factors that we could not easily control for, are taken care of by using this proxy variable. An important condition for a valid instrumental variable is that the association between the variable and the exposure of interest is strong and not confounded by other factors (151). The association between blood group and length of storage is evident from the difference in mean storage time found between blood groups (see Figure 11). We also adjusted for hospital, year and indication to clean the association between blood group and storage time from possible confounders.

Figure 11. Blood group and storage time



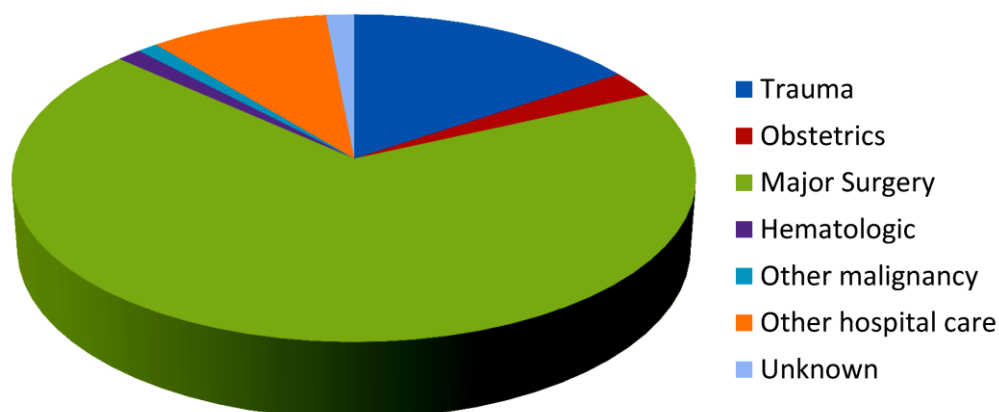
In study 4, we might speculate that the association between risk-score in the donor and ARDS in the recipient is a result of some residual confounding. A thorough investigation in order to identify such a confounder failed. More detailed data both regarding patient characteristics and the time variable between transfusion and outcome is desirable for future research.

## 11. Main findings and implications

### 11.1 Study 1

In study 1 we present the first complete description of the epidemiology of massive transfusion. We provide knowledge about patient characteristics, the main indications for massive transfusion and the prognosis in terms of both short- and long-term mortality. We found a non-negligible incidence of massive transfusion, comparable with severe sepsis (185, 186), and a strikingly high mortality which both emphasize the importance for continued research in this area. We also found a different distribution of indications compared to the ones usually referred to (see Figure 12). The absolute majority of the massive transfusions were administered due to major surgery and only a smaller proportion constituted treatment for trauma or obstetrical complications. This highlights the need for including patients, other than trauma and obstetric, in future research concerning massive transfusion. It also opens up for a discussion about interventions for reducing massive transfusions, such as advances in surgical techniques, the development in using blood saving devices and increasing use of autologous blood transfusions.

Figure 12. Massive transfusion- Indications



### 11.2 Study 2

In study 2 we show a strikingly different risk of death associated with plasma to RBC ratio when we compared analyses with and without taking time into account. This discrepancy suggests that previous observational studies indeed suffered from survival bias. Our main result, showing no effect of high compared to low plasma ratio on mortality in trauma patients, is not necessary evidence for the lack of such an effect. Our study population is small and not powered to detect small but yet



clinically significant differences. However, the lack of association presented in the RCT performed by Holcomb et.al (46) indicates that previous consensus about the benefits of increasing plasma ratio in massively bleeding trauma patients should at least be questioned and further research is needed before it can be considered to be an evidence-based treatment.

### **11.3 Study 3**

In study 3 we show, by using three different analyses approaches, that the length of storage of red blood cells has no association with risk of death. Even when studying patients receiving multiple blood units near expiration date, the risk of death is not increased compared to patients receiving fresher blood. With our large-scale population based cohort, combined with previous results from RCTs, we provide strong evidence for the safety of today's practice. Since concerns regarding potential harmful effects of red blood cells stored for long time still are present, and since a reduction in storage time might threaten the availability of blood products (172) we think that the results from study 3 is of high importance and should alleviate those concerns.

### **11.4 Study 4**

In study 4 we found a strong association between certain donors and the risk of ARDS in transfusion recipients. After reviewing medical records we could not confirm that the ARDS cases actually represent TRALI. The statistical method we used has been shown to be valid for detection of donors carrying hepatitis virus (187). However, our hypothesis that the same model could be used in identifying donors with high risk of causing TRALI in the recipient could not be confirmed. This might partly be explained by TRALI being a more complex condition where probably factors in both donor and recipient, as well as the interaction between those factor are needed for the condition to emerge (188), and partly by our risk-score not being discriminatory enough to identify true high risk donors. At this moment we do not know if a donor-related factor is binary or have a dose-response effect. We also lack knowledge about this possible factors penetration into a clinical condition in the recipient. The association found can at the moment neither be dismissed nor explained. We believe that the donors identified by our method, indeed have some risk factor that is involved in TRALI but until the etiology of TRALI is better understood, this method fails in being a way of studying the condition.

## 12. Conclusions

- Massive transfusion is not an extremely rare event, the incidence being comparable with severe sepsis.
- The prognosis for patients receiving massive transfusion is poor with an overall 5- year survival rate less than 50%.
- The main indication for massive transfusion is major surgery. Massive transfusion due to trauma and obstetrics constitute a smaller part of the cohort.
- The time factor is highly important when studying a possible effect of plasma ratio on mortality.
- Previous research showing beneficial effects of high plasma ratio might partly be explained by survival bias.
- There is today no strong evidence for a beneficial effect of high plasma ratio in bleeding trauma patients.
- There is no association between the storage time of red blood cells and mortality. This is true independent of the indication for transfusion.
- Certain blood donors are associated with a higher risk of ARDS in the recipient. However, a clear temporal association between the transfusion and the diagnosis is lacking.
- Further knowledge about TRALI etiology is mandatory before a statistical algorithm for identifying risky donors can be used.

## **13. Future perspectives**

### **13.1 Blood replacement**

It is well known and accepted that blood transfusion is a treatment associated with certain risks that might influence the outcome of patients. Even though there is ongoing work to find replacements for red cell transfusion, amongst them the development of free hemoglobin solutions and synthetically produced perfluorocarbons (189), these substitutes are still associated with significant adverse effects and a safe product available for clinical use lies far ahead in the future (190). For plasma and platelets, freeze-dried products are under development and are subject to clinical trials (191, 192). Fibrinogen and factor concentrate as an alternative to plasma in major bleeding might be an alternative, but the evidence is weak (193), and even though higher fibrinogen levels are achieved by administering fibrinogen concentrate, there is still no obvious and safe replacement fluid for the volume lost. For this reason blood transfusion, as whole blood, blood components or as a combination of products, is still the mandatory treatment for massive bleeding, and all efforts in optimizing the protocols and reducing risks associated with it, are of high value both for the patients involved as well as for society.

### **13.2 Prediction of massive transfusion**

Almost all studies regarding blood transfusion and the proportion of components have been performed among patients receiving massive transfusion. The definition excludes patients with critical bleeding that do not receive 10 units of red blood cells, because of early death or because of early bleeding control. This is a natural consequence of the retrospective definition currently used but creates considerable obstacles in detecting beneficial interventions (51, 194, 195). Therefore future studies should focus on the possibility to predict massive bleeding at an early stage. Early identification of patients who will need massive transfusion will allow them to be included in coming studies, enable detection of interventions that reduce the need for massive transfusion and avoid unnecessary transfusions in patients who will not bleed massively. A simple predictive score, including factors easily detected at admission, with both high sensitivity and specificity is highly desirable.

### **13.3 Extending cohorts**

The vast majority of studies on massive transfusion have focused on a trauma population. In this thesis we present detailed epidemiology of patients massively transfused, showing that the majority of them is due to major surgery and only a smaller proportion due to trauma. Since trauma-associated hemorrhage is known to create a specific coagulopathy, named ETIC (24), results from trauma populations are not obviously generalizable to other populations. There is an urgent need to establish appropriate strategies for blood transfusion in patients bleeding due to major surgery and

indications other than trauma and development of such strategies require research with extended inclusion criteria.

### **13.4 Storage time, morbidity and efficacy**

Although the storage of RBC does not seem to affect mortality, there is still certain paucity in data regarding the possible risk that storage time affects morbidity or reduces the efficacy of the transfusions administered. In a RCT performed by Dhabangi et al (127) the time to clear elevated lactate was compared between children receiving RBC stored for short and long time. They showed non-inferiority of RBC stored for longer time which should be interpreted as evidence of equal efficacy. Similarly a RCT among patients undergoing cardio-vascular surgery showed no difference in tissue oxygenation between patients receiving fresh versus old blood (196). However, these studies were performed in specific subgroups of patients and more studies on efficacy are needed.

### **13.5 Whole blood transfusions**

There are some indices of a beneficial effect in replacing blood component therapy with Whole Blood (WB) transfusions (197). In vitro data indicates that WB might achieve hemostasis better than reconstituted whole blood with components (198) and in clinical settings produce less coagulopathy (199). In a trial by Cotton et al., comparing WB to RBC and plasma in a ratio 1:1, WB reduced the total need of transfused units (200). In certain military settings, WB is now the first choice when resuscitating in cases of traumatic hemorrhage (201). Usage of WB makes the discussion about ratios redundant but on the other hand practical obstacles regarding blood processing, storage and compatibility testing emerge. Also, as previously emphasized, military trauma patients might differ considerably from other trauma patients and further research in civilian settings is warranted.

### **13.6 Long-term outcomes**

In study 1 we show that mortality among transfused patients is highly variable according to the indication. Overall the mortality is high, >50% are dead within 5 years. Patients transfused for obstetrical reasons had a markedly high survival compared to all other groups. This, in combination with young age at transfusion and very seldom any comorbidity distinguish them and make them extremely suitable for studying long term effects of blood transfusion. Concerns of increased risk for lymphoma in patients previously transfused (202, 203) could be investigated in patients transfused for obstetrical reasons using SCANDAT2 both for identification of transfused patients and follow-up regarding outcome. Since both transfusion for obstetrical reasons and especially lymphoma are rare events, such a study would require both a large cohort and an extended follow-up time to be able to detect any increased risk, if such exists.

### **13.7 Expanding SCANDAT2**

After completing study 2, Holcomb et al. (46) presented a multi-centered RCT comparing high to low plasma ratio in trauma patients with significant bleeding. They did not find any difference in mortality at 24 hours or at 30 days even though the proportion of patients dying from exsanguination was significantly reduced in patients randomized to high plasma ratio. The comparison was made between patients receiving plasma to RBC in a ratio 1:1 and 1:2. The trial included 680 patients, a group too small to detect the predefined difference considered to be clinically significant. This highlights the obstacles that surround the performance of RCTs in trauma patients and emphasizes the role for well conducted observational studies that enables inclusion of larger sample sizes. The current SCANDAT2 only collects on which day a transfusion has been administered and not the exact time. This prevents the use of SCANDAT2 in assessing effects of plasma ratio on mortality in a correctly time-dependent manner. In the future, the incorporation of administration time of the transfusion in the database, would allow large scale observational study of the effects of plasma ratio. With larger sample size a more extreme categorization of plasma ratio would also be possible, with the advantage of detecting a possible critical point regarding plasma amount, if such a point exists.

SCANDAT2 provides reliable data on hard outcomes such as death and this thesis primarily focuses on different aspects of transfusion and the mortality risk. However, there is also a need for assessing the effect of transfusion on morbidity. Future incorporation of quality-care-registers, as for example the Swedish Intensive Care Register (SIR), into the SCANDAT2 database, would enable such studies. In SIR important measures regarding respiration, hemodynamics, coagulation and renal function is recorded daily (204) and the association between transfusions administered and changes in those parameters would allow for important evaluations. Although concordant with studying plasma to RBC ratios, morbidity studies require knowledge about the exact time of the transfusion in order to establish the temporal relationship between the transfusion and its possible adverse effect.

### **13.8 Ratio vs goal-directed therapy**

In our study of plasma ratio, we could not find any difference between those receiving a ratio above or below 0.85. Our cohort consisted of trauma patients managed at one regional center and the variation of plasma ratio was limited. The overall perception in clinical practice today is that high ratio is beneficial and therefore the achieved ratio will probably be quite similar among massively transfused patients. In future research it would therefore be desirable to compare plasma ratio near 1:1 with other goal directed strategies. Viscoelastic tests (TEG®, ROTEM®) as a method of measuring the coagulation bedside and as a method of guiding transfusion therapy, has been proposed as a possible way to improve outcome in massively bleeding patients (205) but no clear evidence for it

exists (206, 207). There are studies comparing the use of viscoelastic tests with the use of standard coagulation tests (208, 209), but very few studies compare therapies based on viscoelastic tests with a ratio driven therapy (210). Since the group of massively bleeding patients includes a high variety of patients with different ages, comorbidities and indications and would therefore presumably benefit differently from various transfusion strategies, further studies that include comparison with individual based therapies are desirable.

### **13.9 TRALI and TACO**

TRALI is admittedly a condition that is difficult to study mainly due to its rare occurrence, the lack of diagnostic tests to confirm the diagnosis and the absence of a specific diagnostic code in ICD (93). Large-scale patient-registers enable detection of a reasonable amount of cases with the possibility to find donor- and or patient-factors that might contribute to the condition. The general opinion is that TRALI is highly under-reported, and the true incidence is unknown (93). However our data in study 4 indicates that this might be exaggerated, at least in a Nordic setting, since we only identified one definitive case of TRALI among the cases reviewed. Epidemiological research to assess incidence rates is thus warranted. Such research must be based on other methods than diagnostic coding. Identification of TRALI through electronic health record-based screening algorithms has been developed (211) but application in clinical settings has been surrounded with difficulties (212). Therefore other methods, focusing on transfused ARDS patients, might be a feasible approach to detect TRALI cases in the future.

Also, further studies on TACO are desirable. Recent data suggests that TACO might also be part of an inflammatory process (66) and thereby TRALI and TACO might be overlapping conditions. In contrast, other data has identified considerable differences in concentration of inflammatory substances between TRALI and TACO patients, where the former have increased levels of interleukin (IL)-6 and IL-8 (known pro-inflammatory agents) while the latter have increased levels of IL-10 (known anti-inflammatory agent) (213). Anyway, volume overload does not seem to be the only explanation for TACO since more than 20% of diagnosed cases only received one single unit of RBC before the symptoms occurred (214). Identification of possible donor- and patient-related factors for both TRALI and TACO, in register studies, might generate hypotheses that can further be tested in clinical trials. A confirmation of risk factors would in a desirable future enable more individualized transfusion therapies, where patients with high risk of transfusion-related complication could be matched to receive blood with low risk to trigger such an event.

## 14. Svensk sammanfattning

Blodtransfusion utgör idag en utbredd behandling som används vid en rad olika sjukdomstillstånd. Över 100 miljoner blodtransfusioner utförs årligen i världen och patienterna som exponeras för behandlingens möjliga biverkningar är därför många. Denna avhandling fokuserar på den kritiskt sjuka patienten, som både i stor utsträckning är exponerad för blodtransfusion och, genom sin underliggande sjukdom, också har hög risk att utveckla biverkningar av densamma. Det övergripande syftet var att beskriva gruppen av massivt transfunderade patienter och studera vissa delar av transfusionsbehandlingen som har föreslagits kunna påverka patientens utfall. Vidare studerade vi möjligheten att genom medicinska register identifiera det ovanliga men högst allvarliga tillståndet transfusion related acute lung injury (TRALI), som idag utgör den ledande transfusionsrelaterade dödsorsaken. I studie 1 beskrivs populationen av massivt transfunderade patienter i Sverige och Danmark under de senaste decennierna. Vi fann en icke negligerbar incidens av massiv transfusion och den dominerande indikationen för transfusionsbehandling utgjordes av stor kirurgi. Dödligheten bland de massivt transfunderade var hög, både mätt efter 30 dagar och 5 år. Den standardiserade dödligheten (SMR) var 26.2 sex månader efter transfusionstillfället, reducerades med tiden men var fortsatt förhöjd så långt som 10 år efter transfusionshändelsen. I studie 2 studerade vi effekten av kvot mellan plasma och erytrocytkoncentrat hos traumapatienter. I en tidsberoende analys, och i motsats till tidigare observationsstudier, fann vi ingen effekt av hög plasmakvot på patientens överlevnad. I studie 3 undersöktes en möjlig negativ effekt av erytrocytkoncentrat med lång lagringstid. Vi använde oss av tre olika analysmetoder för att uppskatta associationen mellan lagringstid och död hos transfunderade patienter. Genomgående i samtliga analyser fann vi ingen som helst association. I linje med nyligen publicerade resultat från randomiserade studier, visar våra resultat på säkerheten med dagens praxis att lagra erytrocytkoncentrat upp till 42 dagar. Studie 4 genomfördes i syfte att utveckla och testa en ny statistisk metod för att identifiera donatorer med hög risk att orsaka TRALI hos mottagaren. Den statistiska metoden var baserad på diagnosen ARDS hos transfunderade patienter. Vi konstruerade en "risk score" utifrån skillnaden mellan observerade och förväntade ARDS-fall bland mottagare från varje enskild donator. Genom denna risk score valde vi sedan patienter för journalgenomgång, vilket resulterade i ett enda identifierat TRALI-fall och vår slutsats är därmed att vår statistiska metod för närvarande inte kan användas för att identifiera och vidare studera TRALI.

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